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# The effects of impurities in papain on triarylmethane photochromism

Jon H. Austin

# THE EFFECTS OF IMPURITIES IN PAPAIN ON TRIARYLMETHANE PHOTOCHROMISM

by

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B.U.S. University of New Mexico

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Coordinator, Graduate Program

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Jon H. Austin

Submitted to the Photographic Science and Instrumentation Division on partial fulfillment of the requirements for the Master of Science degree at the Rochester Institute of Technology

## ABSTRACT

The effects of the enzyme papain on triarylmethane photochromism were investigated. The interaction between papain solutions and triarylmethane dyes was measured using difference spectroscopy and relative equilibrium constants were calculated. The effects of papain on Malachite Green photochromic solutions were also measured. Both crude and commercially available purified papain were tested. Crude papain bleached triarylmethane dyes and reduced fatigue in triarylmethane photochromic solutions. Purified papain did not bleach the dyes and did not have a significant effect on fatigue in photochromic solutions. It was not as soluble in the alcohol based solvent as the crude enzyme was. It is believed that an impurity is responsible for bleaching triarylmethane dyes and reducing fatigue in triarylmethane photochromic systems.

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# INTRODUCTION

Triarylmethane dyes are bleached by cyanide, bisulfite and other ions to form compounds that are photochromic in solutions of polar solvents. These compounds are commonly referred to as the leuco form of the dye. When solutions of these compounds are exposed to ultraviolet radiation, they ionize to give the colored dye cation. Maximum density is generally achieved in approximately 10 to 50 microseconds, the time being dependent on the intensity profile of the source. The reverse reaction occurs when the exposing source is removed (see figure 1), that is, the color fades. The rate of the fade reaction is thermally dependent and follows second order kinetics. [1] [2]

The photochromic response of triarylmethane systems may be characterized by two parameters: sensitivity (the initial density achieved for a given exposure) and fade time to half maximum density. These parameters vary with the constituents of the system. The more polar the solvent system is, the more stable the colored dye cation becomes. Consequently, fade time increases. If the solvent system is too polar then the triarylmethane leucocyanide solubility is reduced. Triarylmethane leucocyanide are not soluble in water so photochromic solvent systems are typically alcohol

based. Organic solvents or water are added to adjust the polarity. Another important ingredient is an excess of bleaching anion, added as the salt. This reduces the fade time. Other compounds are added in some cases but this is the basic photochromic system. There are some problems with these systems. After a solution has been cycled from colorless to colored and back several times it fatigues. The sensitivity decreases and the fade time increases. This investigation concerns the effect of the enzyme papain on fatigue.

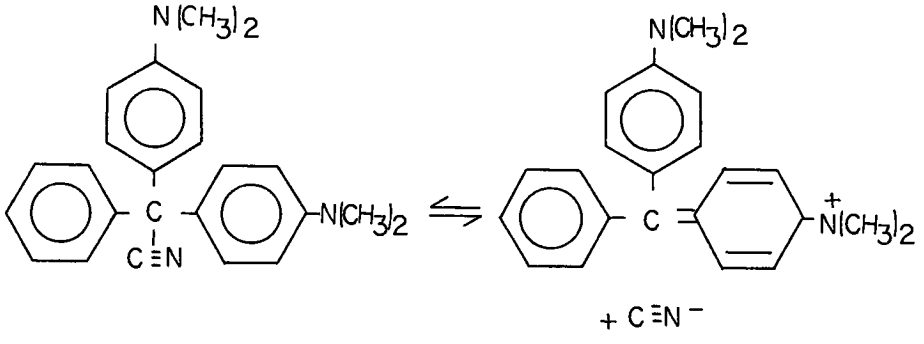



Figure 1

Malachite Green leucocyanide photochromic reaction

Papain is a proteolytic enzyme derived from papaya latex. It is a single folded polypeptide chain 212 amino acids long. The biological activity site is located at the

only free sulfhydryl group on the enzyme (cysteine number 25). (See figure 3). X-ray diffraction studies have shown that six other sulfhydryl groups form disulfide bridges, crosslinking the enzyme. [3] The enzyme alone will not catalyze protolysis. It must be activated by cystein or cyanide.

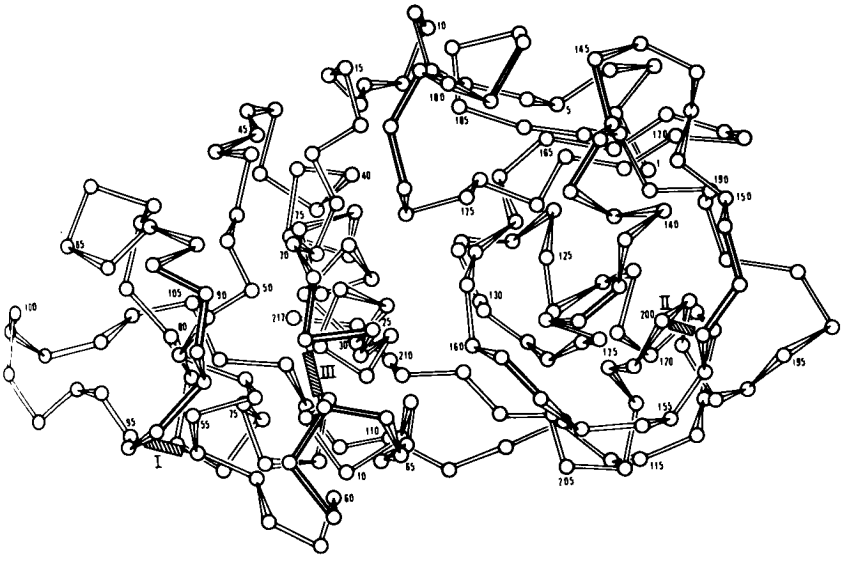


Figure 2

Three dimensional structure of papain

(Courtesy of Dr. J. Drenth,

with the permission of Macmillan (journals), London)

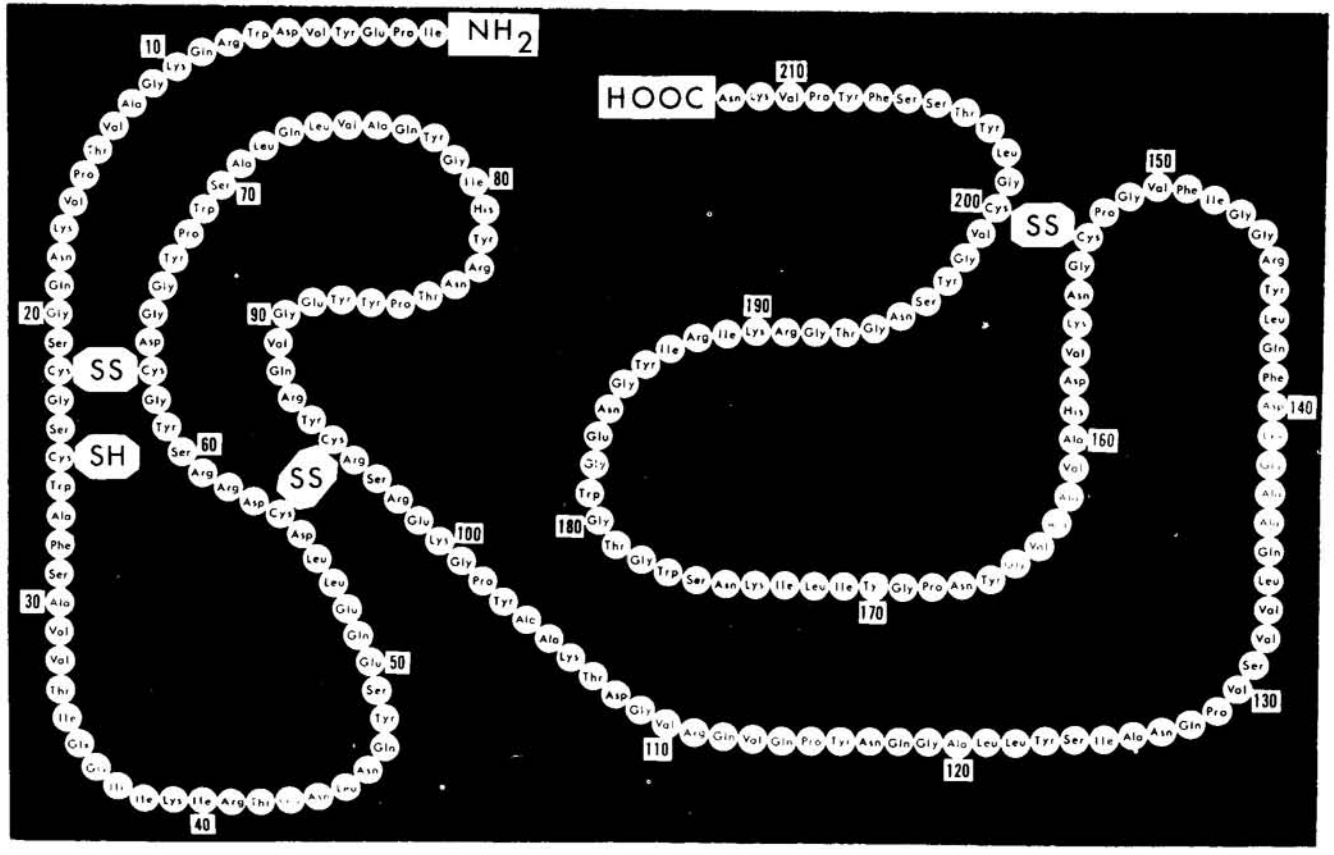



Figure 3
Amino Acid Sequence of Papain
(Courtesy of Dr. J. Drenth,
Permission MacMillan (Journals), London)

In 1964, a solution to the fatigue problem was discovered. Allinikov of the Air Force Materials Laboratory reported that the enzyme papain significantly reduced fatigue. [4] [5] Photochromic solutions which normally showed fatigue in a few cycles could be made to respond without fatigue for over fifty cycles. Allinikov's inspiration to investigate the effects of enzymes on photochromism came from contemporary research on photochromic enzymes. [6] Tests of available enzymes revealed that papain was photochromic but sensitivity was low. Allinikov knew that enzymes catalyze biological reactions. He decided to test the hypothesis that they also catalyze photochromic reactions. Papain was tested in solution with commercially available dyes. Results demonstrated that triarylmethane dyes were bleached by papain to make photochromic solutions. Further tests showed that the rate of the fade reaction was increased and that fatique was reduced when papain was added. Subsequent work on dye-enzyme photochromic systems showed that denatured papain was more effective in reducing fatigue than natural papain. [7] [8] Other enzyme preparations (urease, glucose oxidase and serum albumin) were also found to reduce fatigue. Their reactions appear to be similar to the reactions of papain.

These results are of interest in light of what is known about enzyme chemistry. Enzymes are catalysts that facilitate specific biological reactions. It is unusual for several enzymes whose biological functions and sources vary so widely to undergo such similar reactions. The fact that denatured papain prevents fatigue better than the natural enzyme is also unusual. Enzymes depend on their three dimensional structure for biological activity. This structure is destroyed by denaturation. Kropp, Windsor, Brake and Moore [7] hypothesized that sulfhydryl groups on cysteine residues in the polypeptide chain of the enzyme were involved. This seems reasonable since there is only one free sulfhydryl group on the enzyme in its biologically active conformation. The other six sulfhydryl groups on the enzyme are tied up in disulfide bridges. (See figure 3). It seemed possible that these sulfhydryl groups were liberated when the enzyme was denatured. Kropp, Windsor, Brake and Moore tested this hypothesis by blocking the sulfhydryl groups on the enzyme with p-chloromecuribenzoate. Photochromic solutions prepared in this fashion exhibited a marked reduction in fade rate after only a few cycles. These investigators concluded from this evidence that sulfhydryl groups on papain were involved in the interaction with triarylmethane photochromic compounds to reduce fatique. These experiments are not detailed in the report and controls are not mentioned. Kropp, Windsor, Brake and

Moore also reported that other compounds containing sulfhydryl groups failed to reduce fatigue. Levin studied other proteins, small polypeptides, and cysteine, all containing sulfhydryl groups. It was reported that they did not reduce fatigue. [9]

The investigation detailed below began as an attempt to correlate the experimentally determined equilibrium constants for the bleaching reaction between papain and triarylmethane dyes and the fatigue reducing properties of papain in triarylmethane photochromic solutions. Relative equilibrium constants for the reaction between the bleaching species in the crude papain preparation and the dyes were determined. However, no bleaching reaction occured between purified papain and the dyes investigated. The emphasis of the photochromic fatigue testing phase was then changed. The effects of crude papain on triarylmethane photochromic solutions were compared with those of purified papain. The purified papain had no visible effect on fatigue. Crude papain reduced fatigue. The effects of p-chloro-mercuribenzoate in Malachite Green photochromic solutions were studied in the absence of papain. It was found that the fade rate of photochromic solutions was greatly reduced by p-chloromercuribenzoate. From this evidence it is concluded that some impurity present in crude papain and in the samples of papain used by previous investigators is responsible for bleaching triarylmethane dyes and reducing

fatigue in triarylmethane photochromic solutions.

# EXPERIMENTAL

Difference spectroscopy was used to investigate the interaction between papain and triarylmethane dyes in aqueous solution. This technique is commonly used in biochemical investigations to determine equilibrium constants for enzyme catalyzed reactions that result in changed in absorbance in the visible or ultraviolet regions of the spectrum. Difference spectroscopy is well suited to the investigation of papain's reaction with triarylmethane dyes because of the resulting color change of the dyes. Two compartment spectrophotometer cells were used in a Beckman model 25 double beam spectrophotometer. In the reference cell buffered solutions of dye and enzyme were placed in separate compartments. In the reaction cell dye and enzyme were placed in the same compartment. The other compartment contained buffer alone. Any deviation recorded by the instrument was a result of a change in the absorption spectrum of the solution in the reaction cell. (see figure 4).

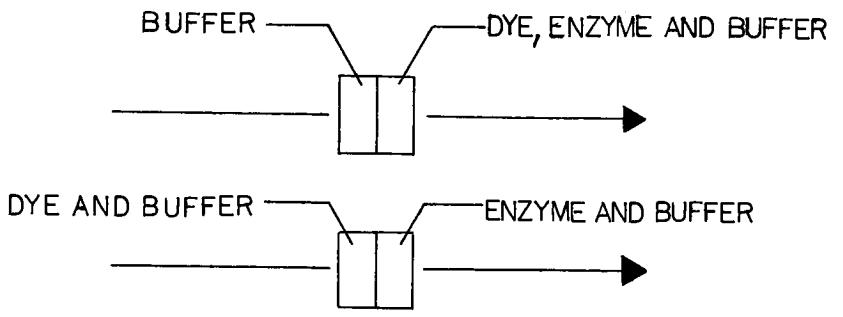



Figure 4

Two compartment cells for difference spectra

Difference Spectra Procedure:

1). Standard papain solutions were mixed in distilled water and commercially available buffer concentrates at pH=7 and pH=10. Buffered solutions were used because the properties of both the enzyme and the dye are pH dependent. The dye becomes colorless at both high and low pH. The solubility characteristics of papain change with pH. Papain concentrations varied from 0.1 to 0.2 mg/ml.

2). Concentrated dye solutions ($10^{-3}$ M) were mixed in distilled water. Fuschsin, New Fuchsin and Malachite Green were investigated.

3). The Beckman 25 double beam spectrophotometer was turned on and allowed to stabilize.

4). Two compartment spectrophotometer cells were prepared in the following manner: In each cell one milliliter of buffered papain solution was placed in one compartment. One milliliter of buffer solution alone was placed in the other compartment of each cell.

5). Both cells were then placed in the spectrophotometer. A zero difference line was recorded as a function of wavelength from 700 nm to 200 nm.

6). A micropipette was then used to titrate a one microliter alliquot of concentrated dye solution into the enzyme solution in the reaction cell and a one microliter alliquot into the buffer solution in the reference cell.

7). The cells were again placed in the spectrophotometer. The difference in absorbance between the two cells was again recorded as a function of wavelength on the same chart paper over the same spectral range.

8). This titration procedure (steps 6 and 7) was repeated until the difference between the two light paths stops increasing, indicating that the enzyme solution was no longer capable of bleaching dye.

Relative equilibrium constants were calculated using the following formula:

$$K_{eq} = \frac{A/E}{([P_0]- A/E)([D_0]- A/E)}$$

where:

A=difference in absorbance between the zero baseline and a particular peak

E=relative difference extinction coefficient (unknown)

$[P_0]$=inital bleaching ion concentration

$[D_0]$=initial dye concentration

$K_{eq}$=relative equilibrium constant (also unknown)

The initial concentration of the bleaching ion (papain) was uncertain because enzyme samples are rarely pure. A value was calculated based on the molecular weight of the enzyme. A computer program was written to solve the above equation with two unknowns. An iterative technique was used. This program performed the following steps to obtain a solution:

1). Input: The input to the computer program consisted of

a). the initial papain concentration

b). beginning value of E

c). an increment which was added to E during the iteration

d). data in the form of matched pairs of A (absorbance difference values) and their

corresponding initial dye concentrations for each alliquot in a particular run.

2). Computation:

a). The program assumed the inital value of E and computed a value of $K_{eq}$ for each dye concentration (typically 5 or 6 alliquots were required) using the equation above.

b). The percent standard deviation in $K_{eq}$ was then computed and stored.

c). The value of E was incremented and new values for $K_{eq}$ were calculated as in step (2a)

d). The value of the standard deviation in $K_{eq}$ was computed and compared to the previous value so that the values of E and $K_{eq}$ which yield the minimum percent standard deviation in $K_{eq}$ could be reported.

In the fatigue phase of the experiment, the characteristics of solutions without papain were compared to the characteristics of solutions with crude papain and solutions with purified papain. Fade time and sensitivity were monitored. The effects of p-chloromercuribenzoate on photochromic solutions were also investigated.

Malachite Green photochromic solutions were prepared by the following procedure: 0.0050 g of Malachite Green leucocyanide (Prepared by R. Bayley [10]) and 0.0050 g of

sodium cyanide were dissolved in four milliters of dimethyl sulfoxide (DMSO), in a 100 ml volumetric flask. Heating was required to dissolve the mixture. Next, 70 ml of methanol (MeOH) were added to this mixture and this solution was diluted to 100 ml total volume with distilled water. Papain (when added) was dissolved in the distilled water fraction. Amounts varied (see table 1). When para-chloro-mercuribenzoic acid was added, it was dissolved in the solution after the methanol was added. The pH was adjusted with sodium hydroxide to pH=10.

Photochromic solutions were tested in an instrument designed and built by Bayley [10] and modified by Stanzioni. [11] The instrument is constructed using two Strobonar model 202 (40 joule) xenon strobe units, a monochrometer and a detector and logarithmic converter connected to a chart recorder as shown in figure 5. The strobe units expose the sample momentarily saturating the detector. When the detector recovers, the density of the test solution is recorded as a function of time on the chart recorder. See figure 8 for a sample of photochromic test data. The instrument was calibrated by inserting a 0.5 neutral density filter into the sample chamber. Photochromic solutions were placed in a quartz cell. The pathlength along the exposing axis was two millimeters to insure uniform exposure. The pathlength along the axis for the measurement of density was one centimeter.

It was determined that the wavelength of maximum absorbance in this solvent system is 610 nm. All photochromic tests were done with the monochrometer set at this value.

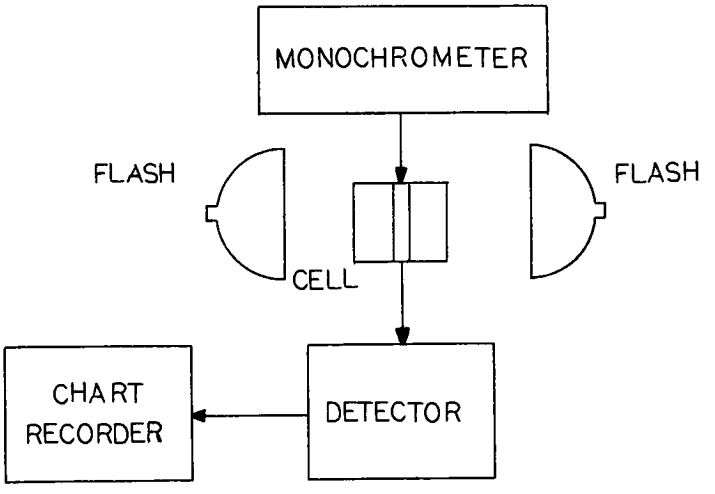



Figure 5

Exposing unit

# RESULTS

Difference spectra were used to measure the interaction between various dyes and papain, both crude and purified. Crude papain was obtained from Difco Laboratories. Purified papain samples investigated came from Sigma Biochemical Co., and Worthington Biochemicals. Crude papain bleached triarylmethane dyes. Dyes investigated were Malachite Green Fuschsin, and New Fuschin. Maximum differences in absorbance recorded for a crude enzyme concentration of 0.1 to 0.2 mg/ml were typically 0.1 to 0.6. The saturation values for the undenatured enzyme solution were obtained after addition of five or six one microliter alliquots of dye. When the crude enzyme was denatured by boiling, the equilibrium constant for the dye and bleaching species more than doubled. (Compare the equilibrium constants labelled Malachite Green, pH = 7 and Malachite Green*, pH = 7 in table 1) Purified papain from either Sigma Biochemical or Worthington Biochemical displayed no significant difference in absorbance even at increased enzyme concentrations up to 1 mg/ml. This implies that the equilibrium constant is zero. The three dyes investigated were Malachite Green, Fuschin and New Fuschin.

Several facts come to light when the difference spectrum for a dye is compared with the absorption spectrum. (See figure 6). Negative peaks occurs where the absorption

maxima of the dye occur. This corresponds to the disappearance of the colored form of the dye. A positive peak in the near ultraviolet portion of the difference spectra indicates the formation of a colorless species. This colorless species is presumably the leuco form of the dye.

Note: Some difference spectra in the appendix are upside down. This simply means that the reaction and reference cells were switched in the spectrophotometer so that the curves would fit on chart paper.

Table 1

Table of Equilibrium Constants

| Dye | pH | peak (nm) | $K_{eq}$ | E | % Standard Deviation |
|---|---|---|---|---|---|
| Malachite Green | 7 | 615 | 4.75 | 92590 | 15.58 |
| New Fuschin | 7 | 545 | 2.60 | 71250 | 12.02 |
| Fuschin | 7 | 545 | 4.52 | 51430 | 23.80 |
| Malachite Green* | 7 | 615 | 13.35 | 57390 | 10.62 |
| Malachite Green | 10 | 615 | 4.01 | 103670 | 24.27 |
| Fuschin | 10 | 545 | 16.06 | 54840 | 31.19 |

---

* Denatured enzyme

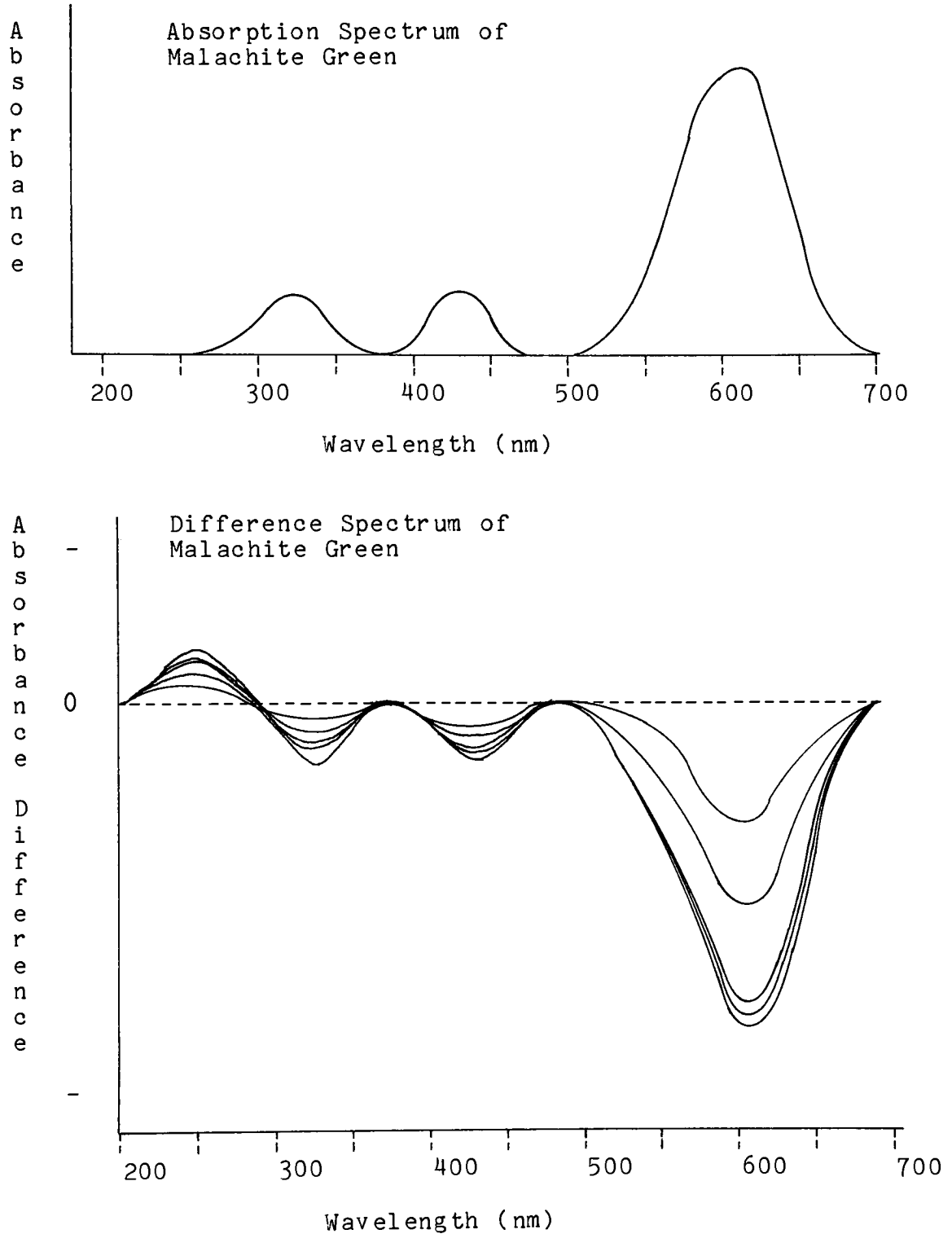



Figure 6

A comparison of the difference
and absorption spectrum of Malachite Green

Malachite Green leucocyanide (MGCN) photochromic solutions mixed according to the procedure given in the experimental procedure section gave initial densities of about 0.5 when exposed in the test cell. Fade times to half of the maximum density (or simply fade time) were 9 to 10 seconds for solutions containing crude papain, 16 to 18 seconds for solutions without papain and ranged from 20 to 28 seconds with purified papain. Fade times for solutions containing p-chloromecuribenzoic acid (p-CMB) were 70 seconds at $2x10^{-5}$ moles/liter and over three minutes at $7x10^{-4}$ moles/liter. The initial density of the solution on the twentieth exposure divided by the density on the first exposure expressed as a percent was chosen as the best test statistic for the photochromic tests. Percent fatigue was calculated according to the following formula:

$$\% \text{ Fatigue} = 100 - \frac{100 \times \text{density on the twentieth exposure}}{\text{density on the first exposure}}$$

Photochromic response experiments demonstrated that purified papain has no significant effect on fatigue. The density of solutions without papain showed 18.6% fatigue after twenty cycles. Solutions with purified papain (0.05 to 0.1 mg/ml) showed 17.6% fatigue after twenty cycles. One sample with purified papain exhibited 10% fatigue. This behavior was not repeatable and may be due to the lack of precision in collecting data from the chart paper. This represents the

effect of the maximum amount of purified papain soluble in the solvent system (70% MeOH, 4% DMSO, 26% water). A supersaturated solution of purified papain exhibited 29% fatigue. Crude papain showed decreasing fatigue with increasing concentration up to 0.5 mg/ml. (See figure 7). At the saturation level of crude papain (0.5 to 1.0 mg/ml) 12% fatigue was exhibited. This is significantly different from the behavior of solutions wihout papain. (99% confidence).

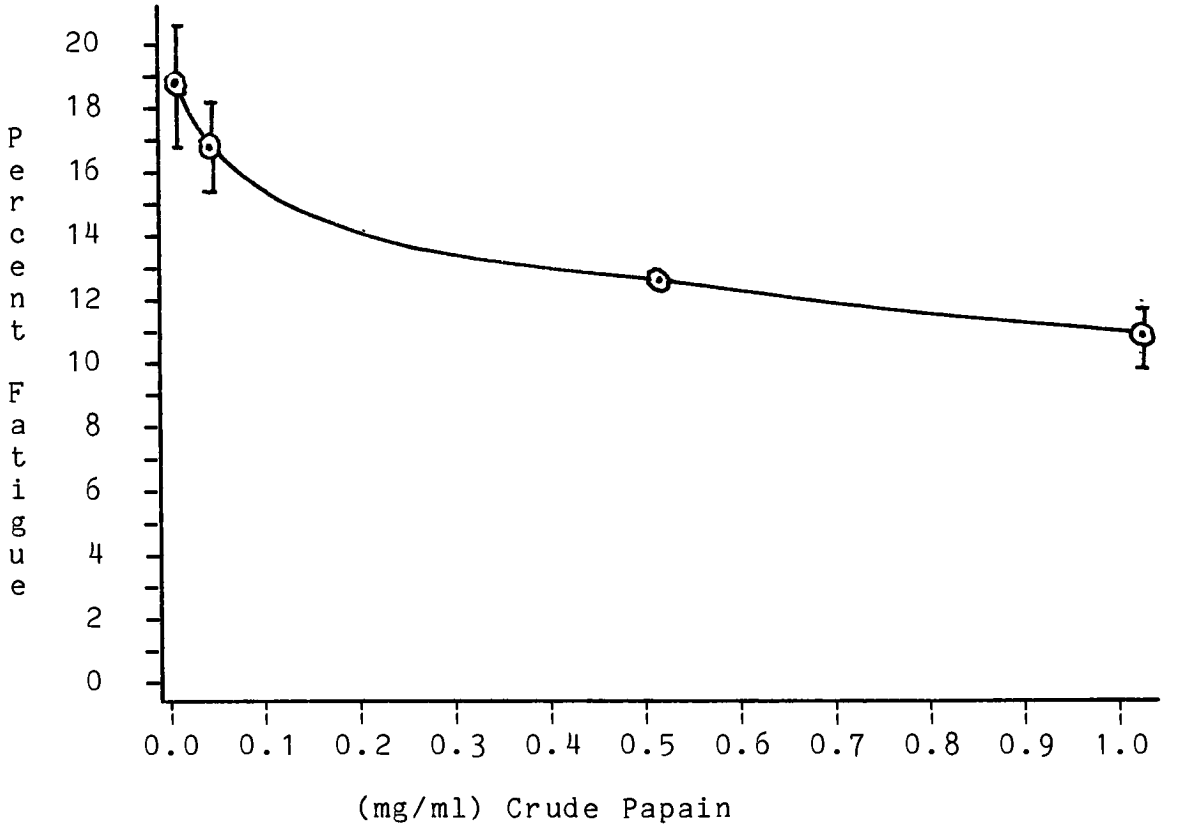



Figure 7

Percent Fatigure v.s. weight of crude protein
(One Standard Deviation error bars)

# FIGURE 8
## Sample Photochromic Test Data

10½ A Malachite Green CN, 50 sec/in, 610nm
Run 1

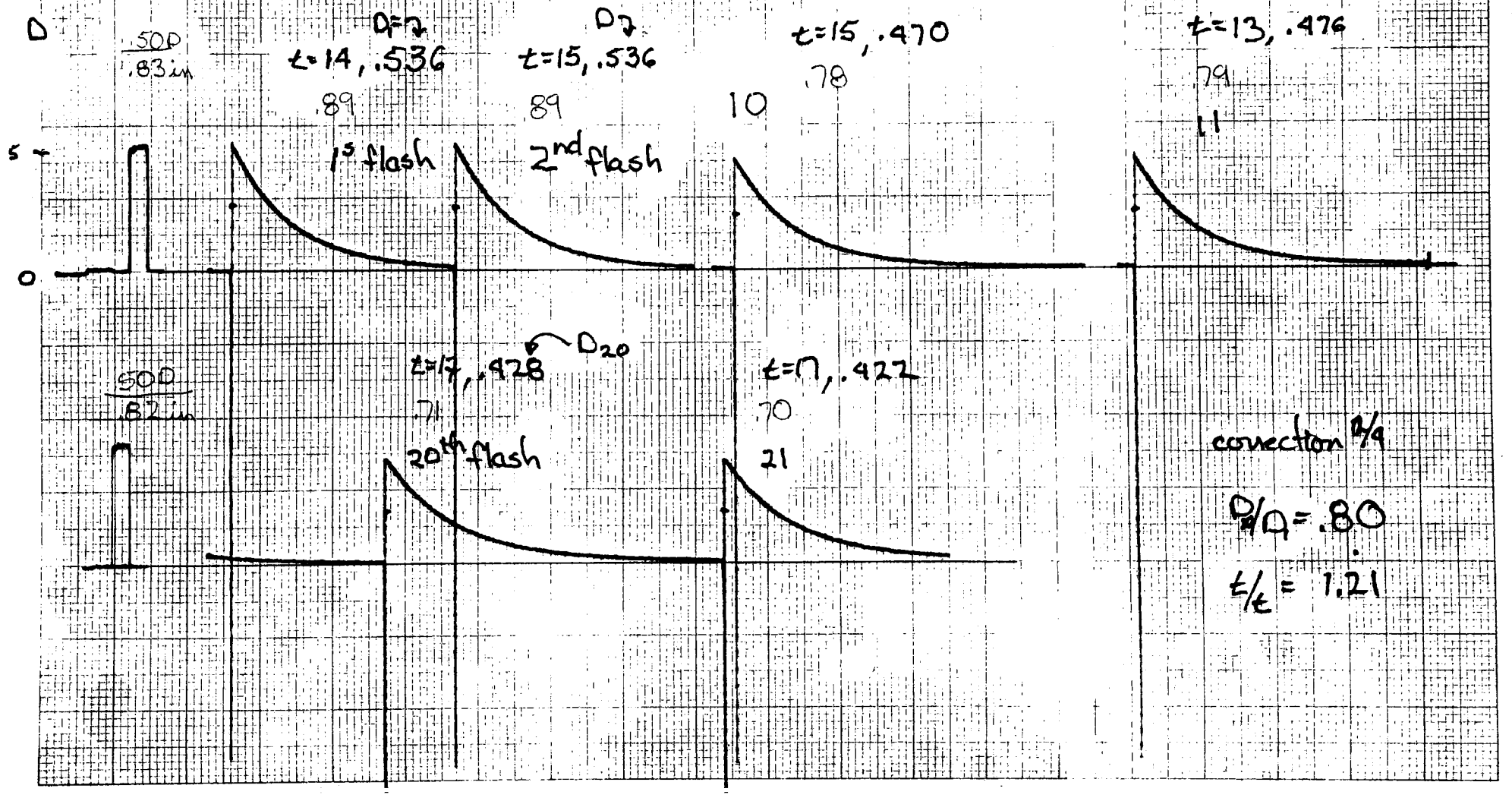


Table 2

Fatigue test data table

Solvent system: 70% Methanol, 4% DMSO, 26% Water

Weights in mg/ml

Solutions without Papain

| # | Wt. MGCN | Wt. NaCN | Wt. papain | % Fatigue | pH |
|---|---|---|---|---|---|
| 1 | 0.051 | 0.050 | | 19 | |
| 2 | 0.051 | 0.044 | | 17 | |
| 3 | 0.051 | 0.044 | | 20 | |
| 4 | 0.051 | 0.044 | | 19 | |
| 5 | 0.056 | 0.052 | | 17 | |
| 6 | 0.056 | 0.052 | | 16 | |
| 7 | 0.051 | 0.051 | | 22 | 10.20 |

Average % Fatigue: 18.6
Standard Deviation: 2.1

Solutions with crude Papain

| | | | | | |
|---|---|---|---|---|---|
| 1 | 0.051 | 0.050 | 1.042 | 13 | |
| 2 | 0.052 | 0.051 | 1.057 | 11 | |
| 3 | 0.052 | 0.051 | 1.057 | 12 | |
| 4 | 0.051 | 0.049 | 0.510 | 13 | |
| 5 | 0.049 | 0.049 | 0.100 | 14 | 9.69 |
| 6 | 0.049 | 0.049 | 0.100 | 16 | 9.69 |
| 7 | 0.050 | 0.050 | 0.095 | 17 | 10.48 |
| 8 | 0.050 | 0.050 | 0.095 | 16 | 10.48 |

See Figure 7.

Solutions with purified Papain

| | | | | | |
|---|---|---|---|---|---|
| 1 | 0.050 | 0.050 | 0.054 | 18 | |
| 2 | 0.048 | 0.048 | 0.083 | 10 | |
| 3 | 0.056 | 0.052 | 0.087 | 21 | |
| 4 | 0.056 | 0.052 | 0.087 | 22 | |
| 5* | 0.051 | 0.051 | 1.0 | 31 | 10.04 |

Average % Fatigue: 17.6
Standard Deviation: 5.3

Table 2 (Continued)

Solutions with p-chloromercuribenzoate (p-CMB)

| # | Wt. MgCN | Wt. NaCN | Wt. p-CMB | % Fatigue | pH |
|---|---|---|---|---|---|
| 1 | 0.051 | 0.050 | 0.252 | + | 10.0 |
| 2 | 0.051 | 0.050 | 0.252 | + | 9.76 |
| 3 | 0.051 | 0.051 | 0.086 | 89 | 10.0 |

---

* This solution was supersaturated with papain. The measurement was made before crystals started to form.

+ This solution faded too slowly to measure.

# CONCLUSIONS

The results of difference spectra experiments, conducted with crude papain, agree with the behavior of papain previously reported. [1] [7] Crude papain bleaches triarylmethane dyes. The crude preparation is a more effective bleaching agent when the enzyme is denatured by boiling. Purified papain does not bleach triarylmethane dyes even when denatured. These results indicate that papain does not interact with triarylmethane dyes. Some impurity must be responsible for the interactions observed. Purified papain has no significant effect on triarylmethane photochromism. Purified papain was present in maximum concentration in this solvent system (0.05 to 0.1 mg/ml depending on the source). In contrast, Kropp, Windsor, Brake and Moore reported that levels of papain up to 10 mg/ml were investigated in a solvent system of 70% methanol and 30% water. This difference in solubility may be due to a difference in pH of the solutions. Kropp, Windsor, Brake and Moore used solutions in the acid pH range. This was possible because bisulfite was used as the bleaching ion. Photochromic solutions in this investigation were in the pH range from 9.5 to 10.5. Because cyanide ion was used as the bleaching ion in this investigation, reduction of the pH into the acid range produced HCN gas.

The effect of papain on photochromic solutions must be due to an impurity. This is probably the same impurity that bleached triarylmethane dyes. Commercial enzyme preparation has improved in the years since 1964 when Allinikov's first work was done. Until now, the papain samples used in investigations of fatigue reduction was prepared by the method of Balls, Lineweaver and Thompson, published in 1937. [12] The purified papain samples used in this investigation were prepared by the more sophisticated method outlined in Methods in Enzymology. [3] The method of Balls, Lineweaver and Thompson uses coagulated papaya latex suspended in toluene as the starting material. The coagulant is separated and suspended in water. The papain is salted out with ammonium sulfate and the solution is cooled to 5 Celsius. Needle-like crystals appear after several days. The method outlined in Methods in Enzymology involves an isolation procedure introduced by Kimmel and Smith and the recrystalization procedure of Balls and Lineweaver. [13] [14] Papain is extracted from the latex with water at pH=5.7. The supernatant liquid from this extraction is adjusted to pH=9 so that insoluble denatured protein can be removed by filtration. The insoluble material is washed with ammonium sulfate solution. Papain is salted out of this solution using sodium chloride and redissolved in cystein solution. The papain cystein solution is cooled to 4 Celsius and the crystals are collected. Then the enzyme

is recrystalized from distilled water using a salting out procedure (NaCl). The recrystalization procedure is repeated several times.

The results of these experiments demonstrate that the fatigue reducing properties of papain preparations are not due to a reaction of the enzyme but are the result of the reaction of some impurity. This impurity is water soluble. It seems reasonable to assume that the impurity is loosely bound to the enzyme. It is possible that when the enzyme is denatured structural changes allow the impurity to escape and interact with the dye.

Future work should include the isolation of the impurity responsible for bleaching the dyes and reducing fatigue. Work on the effects of urease and the other enzymes mentioned would yield information about the types of impurities present in samples of enzymes.

# APPENDIX 1

## List of Materials

1. Crude papain NF VIII, Difco Laboratories, Control 642216
2. Papain, Type IV 2x recrystalized, Sigma Chemical, EC 3.4.22.2
3. Papain, 2x recrystalized, Worthington Biochemical, lot 3126PAP38M980
4. Methanol absolute, J. T. Baker, lot 825921
5. Methanol ACS, Fisher Scientific, lot 772596
6. Methanol ACS, Fisher Scientific, lot 782842
7. DMSO, Fisher Scientifier, lot 755838
8. p-Chloromecuribenzoic acid practical, Aldrich Chemical, lot 031717
9. Sodium bisulfite ACS, Fisher Scientific, lot 730271
10. Sodium cyanide, data not available
11. Malachite green leucocyanide, prepared by Robert Bailey
12. Brilliant blue leucocyanide, prepared by Robert Bailey
13. Crystal violet leucocyanide, prepared by Robert Bailey
14. Fuchsin leucocyanide, prepared by Robert Bailey
15. New fuchsin leucocyanide, prepared by Robert Bailey
16. Malachite green, Allied Chemical 15
17. Pararoseanaline Pfalt and Bauer Inc., no lot number
18. Brilliant Blue - Leucolithosol Blue 6G, Dupont 7-11-75
19. Fuchsin, Pylam Chemical, no lot number
20. New Fuchsin, Pylam Chemical, no lot number
21. Crystal Violet, Fisher Scientific, lot 714037

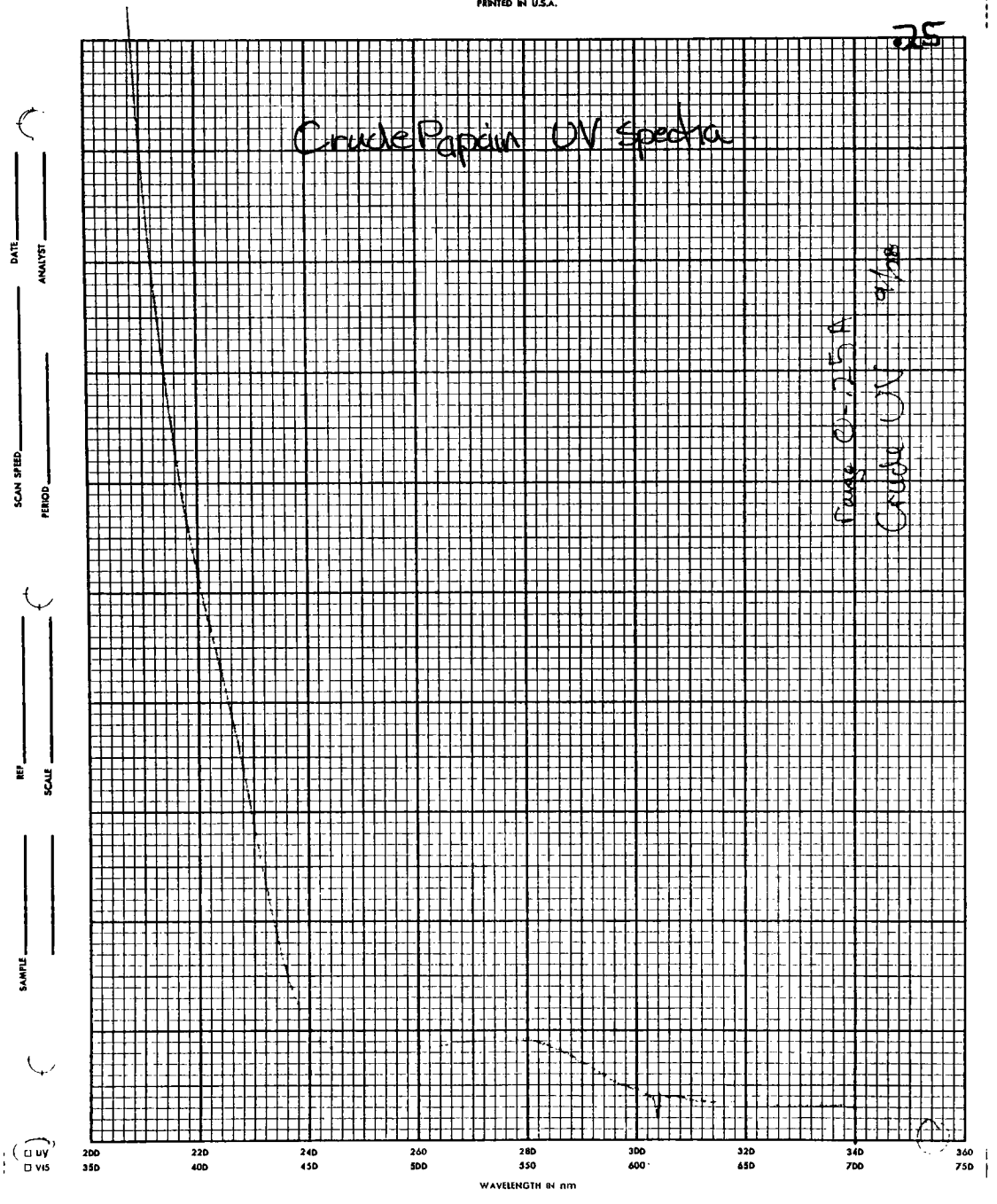
# List of Materials

22. pH 4 buffer, Fisher Scientific, lot 774240
23. pH 7 buffer, Fisher Scientific, lot 775054
24. pH 10 buffer, Fisher Scientific, lot 780774
25. pH 11 buffer, Fisher Scientific, lot 780880

# APPENDIX 2

## Spectra

SPECTRUM OF CRUDE PAPAIN


Crude Papain UV Spectra
Crude UV
Crude 0-25A
DATE
ANALYST
SCAN SPEED
PERIOD
REF
SCALE
SAMPLE
UV
VIS
200 220 240 260 280 300 320 340 360
350 400 450 500 550 600 650 700 750
WAVELENGTH IN nm

SPECTRUM OF PURIFIED PAPAIN

Spectra SIGMA PAPAIN
(V-2 p 30)

Absorption Curve p 48, V-1
274

245 nm at the end

DATE
ANALYST
SCAN SPEED
PERIOD
REF
SCALE
SAMPLE

UV
VIS

220 240 260 280 300 320 340 360
400 450 500 550 600 650 700 750
WAVELENGTH IN nm

BECKMAN INSTRUMENTS, INC., FULLERTON, CALIF. PRINTED IN U.S.A. REORDER BECKMAN PART NO. 580217 86

9/28 +2

Spectrum, Good Papain

200 220 240 260 280 300 320 340
350 400 450 500 550 600 650 700
WAVELENGTH IN nm

SPECTRUM OF MALACHITE GREEN

BECKMAN INSTRUMENTS, INC., FULLERTON, CALIF. PRINTED IN U.S.A. REORDER BECKMAN PART NO. 580217

t=28

9/7/78

D = 1.0

SPECTRUM of -
Malachite green pH=7 buffer
[Dye] = 1.128 × 10⁻⁴ M

DATE
ANALYST
SCAN SPEED
PERIOD
REF
SCALE
SAMPLE

× 2nd in 2ml

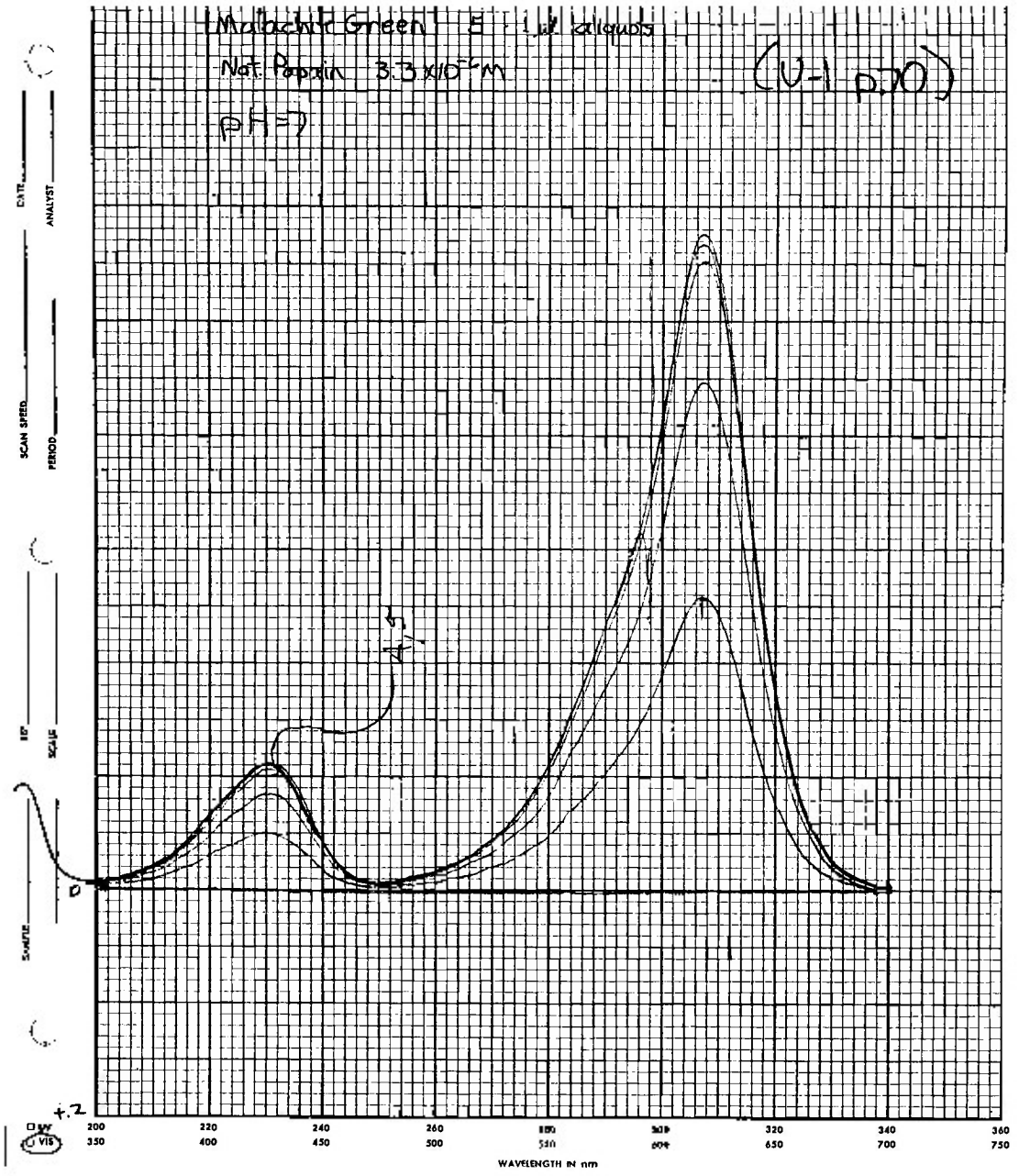
1st in 2ml Buffer

0

UV
VIS

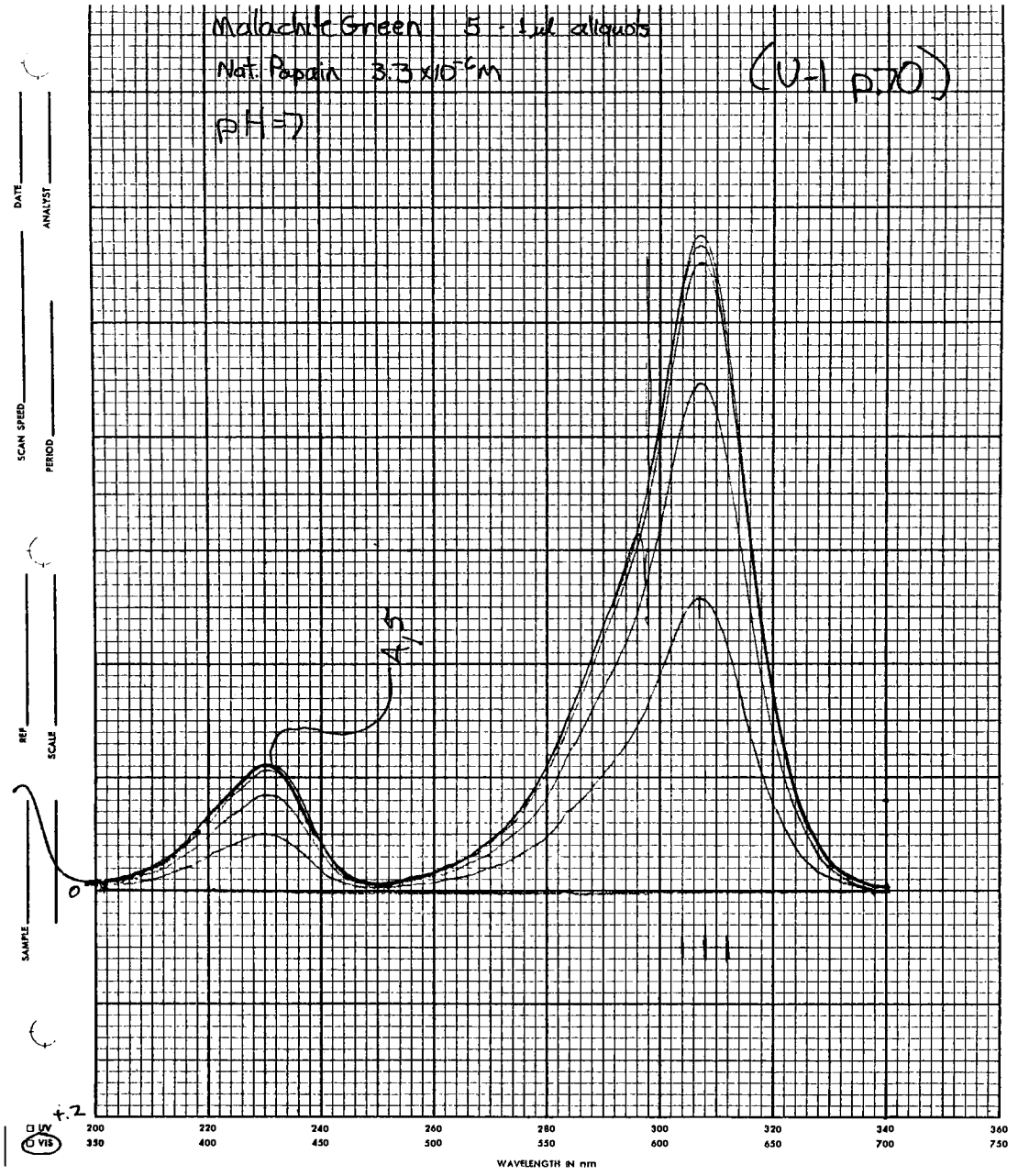
200 220 240 260 280 300 320 340 360
350 400 450 500 550 600 650 700 750

WAVELENGTH IN nm


Malachite Green 5 µl aliquots
Nat. Peptide 3.3x10⁻⁶M
pH=7
(V-1 p.70)
DATE
ANALYST
SCAN SPEED
PERIOD
SCALE
SHUTTER
+.2
UV
VIS
200 220 240 260 280 300 320 340 360
350 400 450 500 550 600 650 700 750
WAVELENGTH IN nm

MALACHITE GREEN - CRUDE PAPAIN DIFFERENCE SPECTRA

pH = 7


Malachite Green 5 · 1 µl aliquots
Nat. Papain 3.3 x 10⁻⁶ M
pH = 7
(U-1 p.70)
DATE
ANALYST
SCAN SPEED
PERIOD
REF
SCALE
SAMPLE
A.5
0
+.2
UV
VIS
200 220 240 260 280 300 320 340 360
330 400 450 500 550 600 650 700 750
WAVELENGTH IN nm

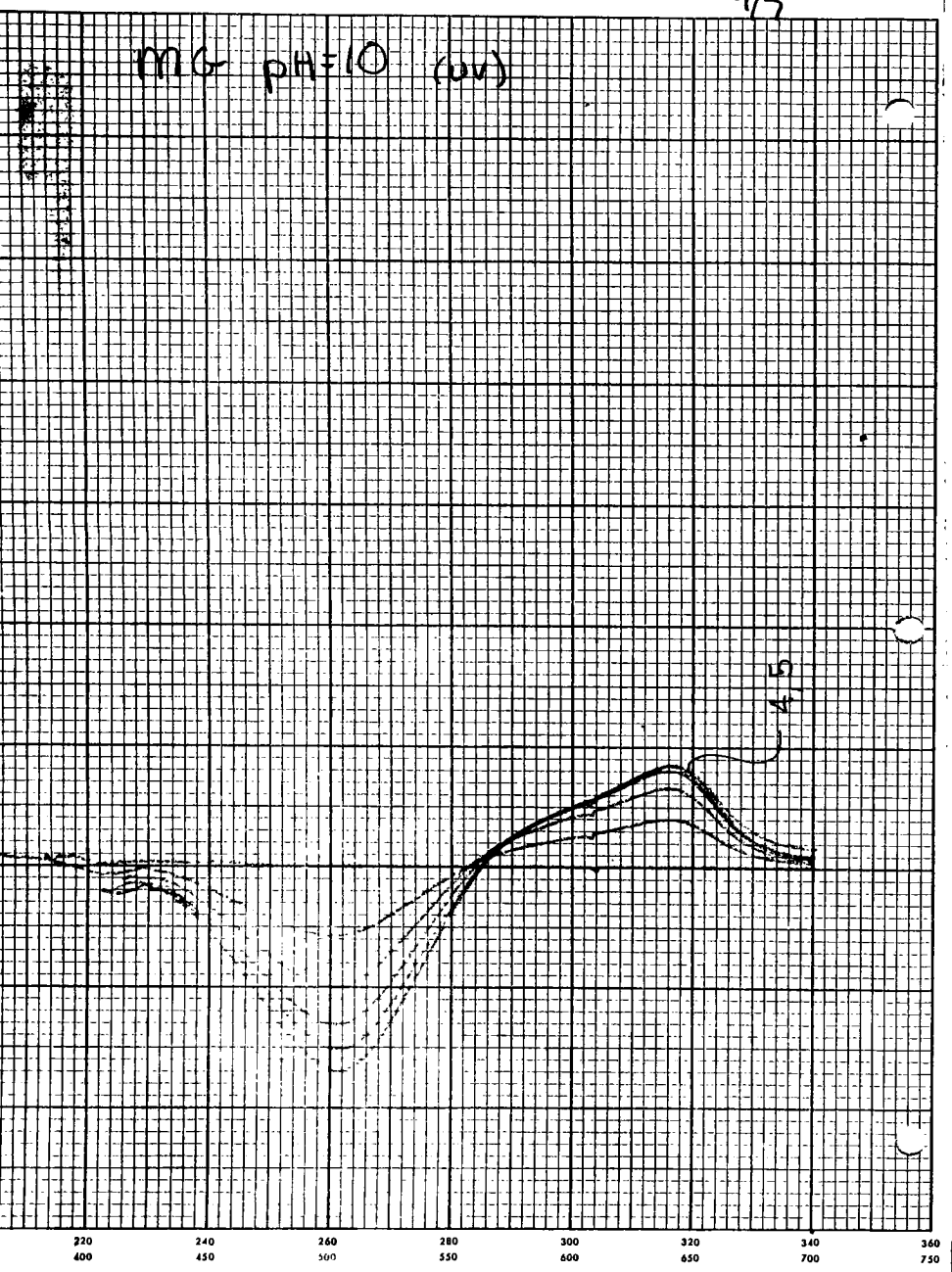
MALACHITE GREEN - CRUDE PAPAIN DIFFERENCE SPECTRA

pH = 10

BECKMAN INSTRUMENTS, INC., FULLERTON, CALIF. PRINTED IN U.S.A. REORDER BECKMAN PART NO. 580217

9/7

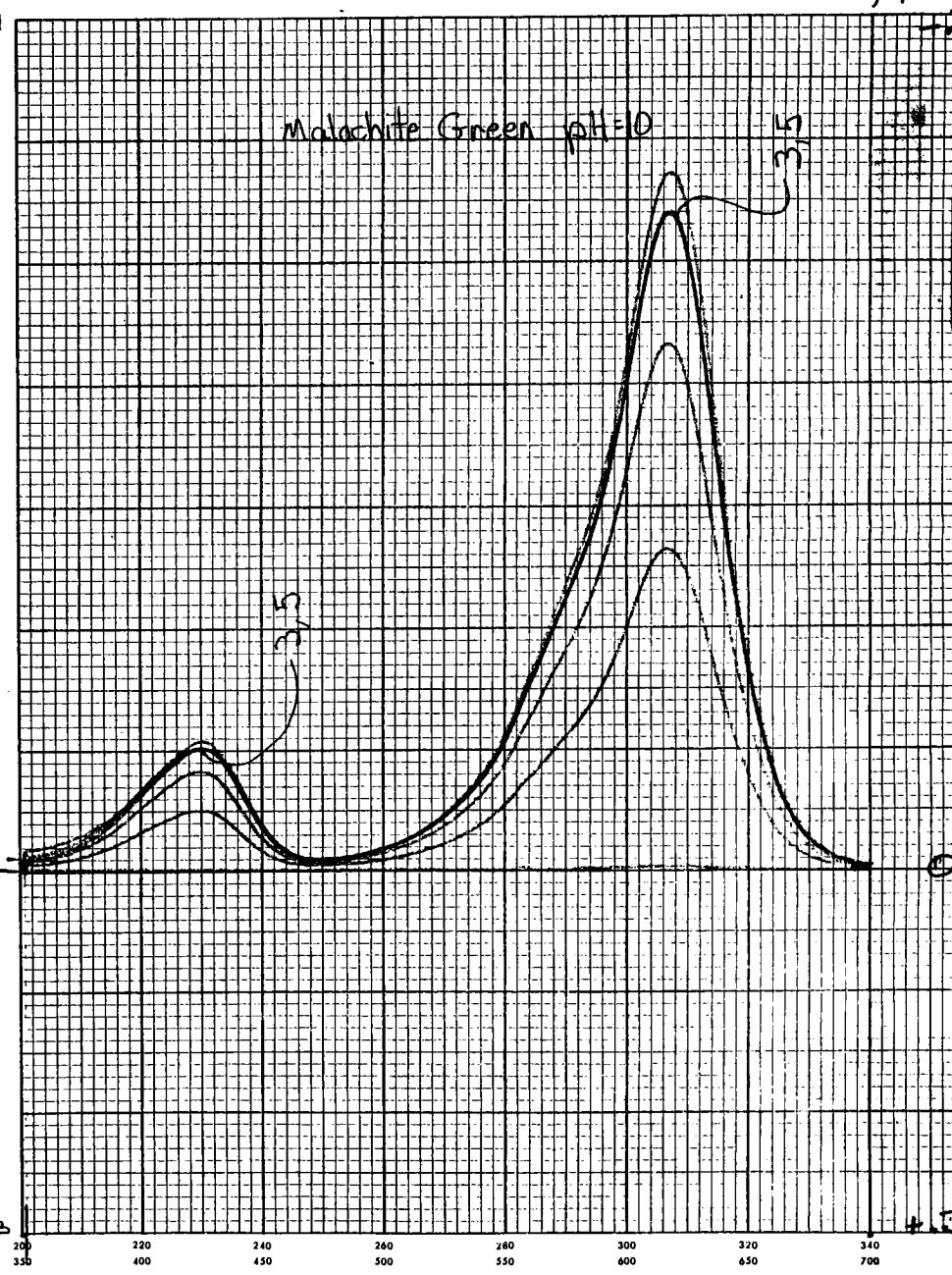
MG pH=10 (uv)




BECKMAN INSTRUMENTS, INC., FULLERTON, CALIF. PRINTED IN U.S.A. REORDER BECKMAN PART NO. 580217

9/7

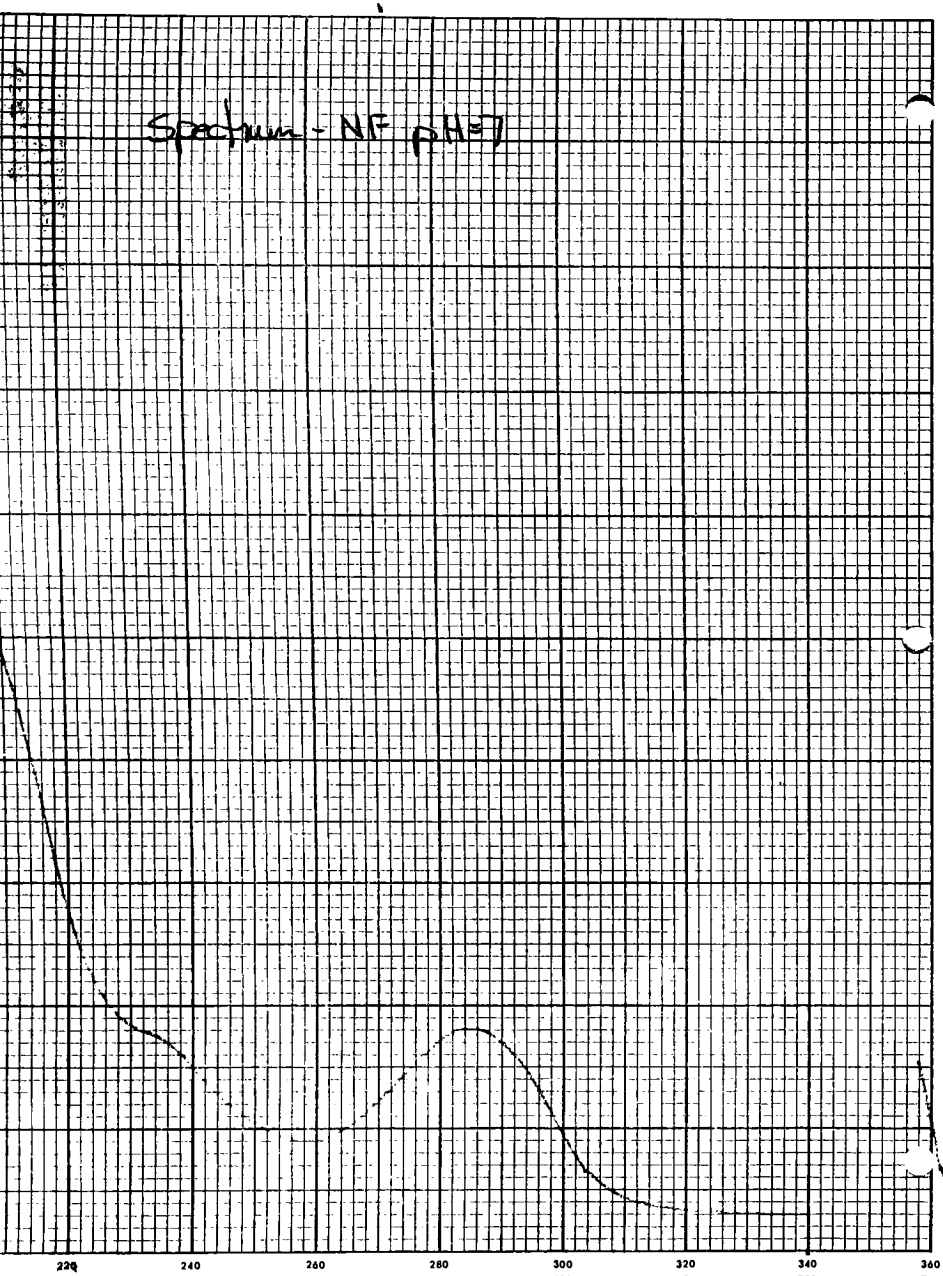
Malachite Green pH=10



SPECTRUM OF NEW FUCHSIN

BECKMAN INSTRUMENTS, INC., FULLERTON, CALIF. PRINTED IN U.S.A. REORDER BECKMAN PART NO. 580217

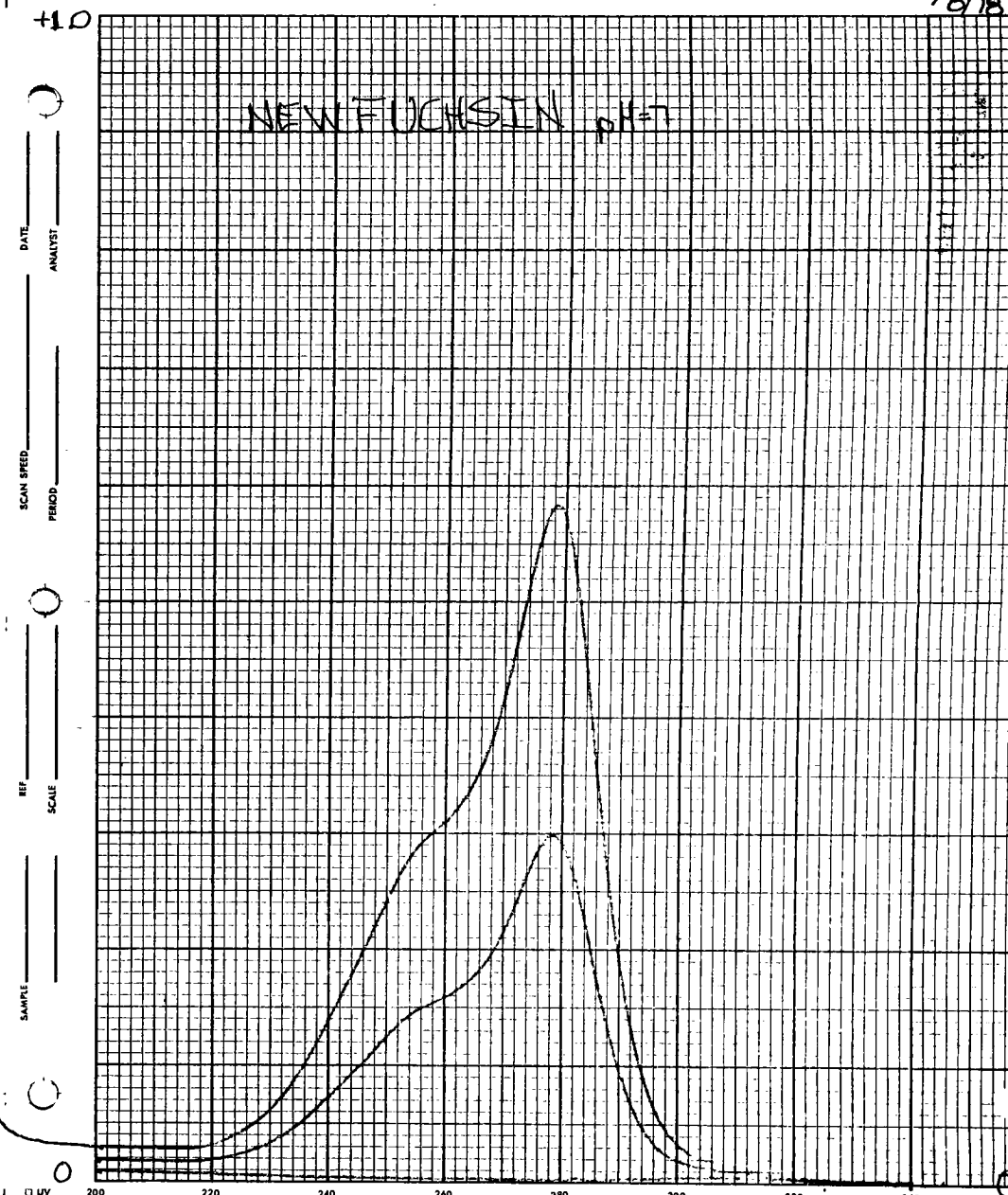
Spectrum - NF pH=7




BECKMAN INSTRUMENTS, INC., FULLERTON, CALIF. PRINTED IN U.S.A. REORDER BECKMAN PART NO. 580217

9/8/78

NEW FUCHSIN pH=7

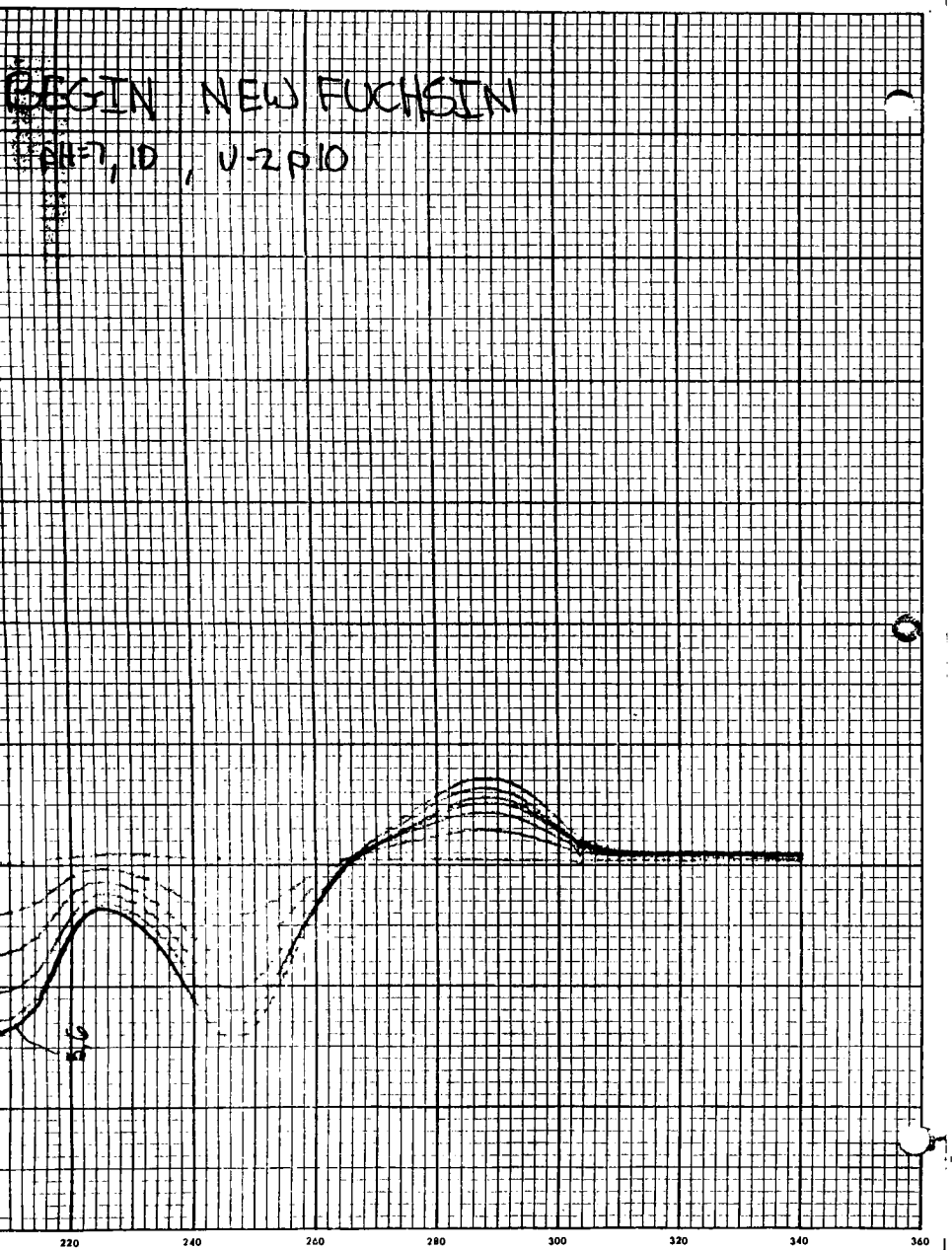


NEW FUCHSIN - CRUDE PAPAIN DIFFERENCE SPECTRA

pH = 7

BECKMAN INSTRUMENTS, INC., FULLERTON, CALIF. PRINTED IN U.S.A. REORDER BECKMAN PART NO. 580217

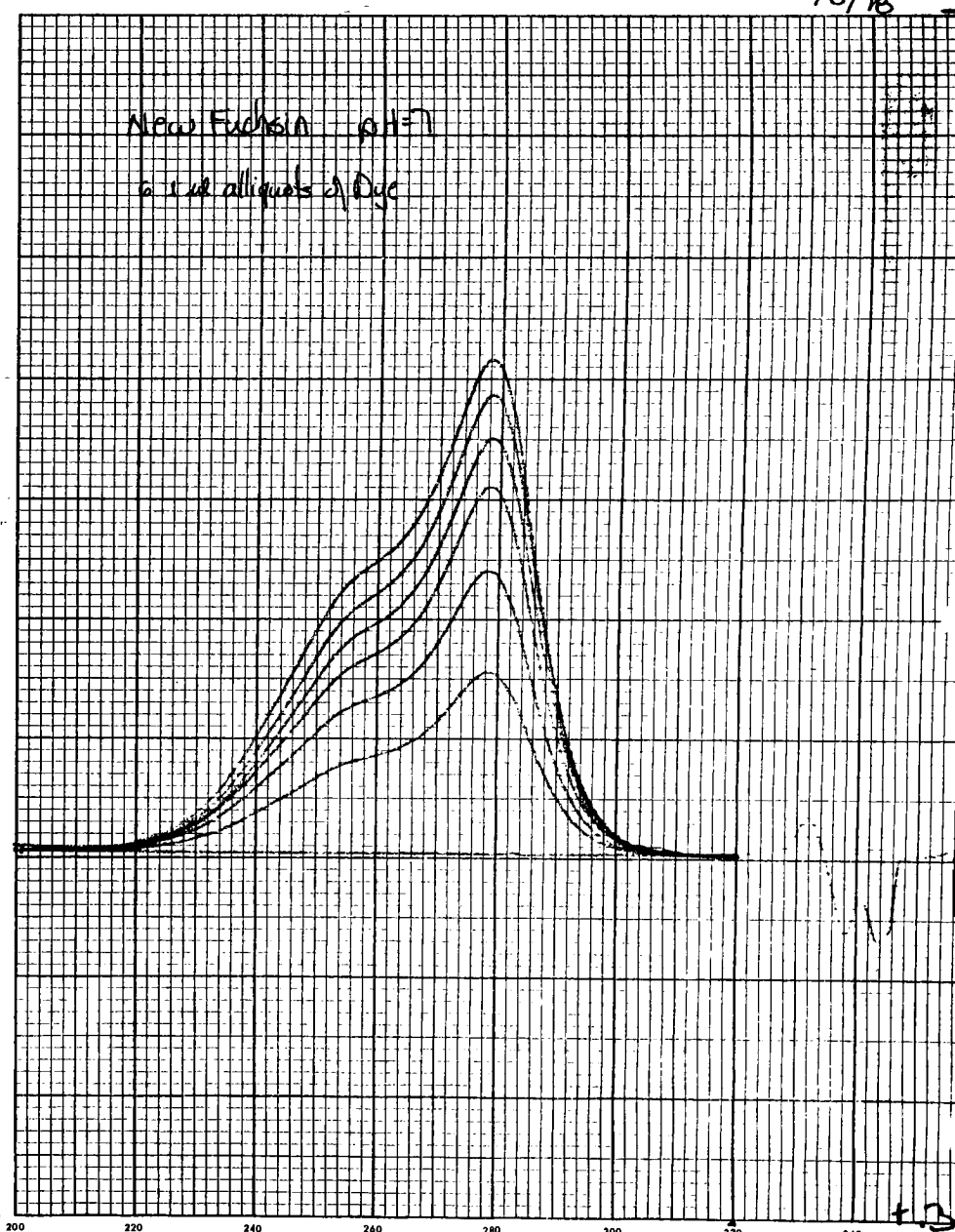
BEGIN NEW FUCHSIN
BR=7, ID, V-2 P10




BECKMAN INSTRUMENTS, INC., FULLERTON, CALIF. PRINTED IN U.S.A. REORDER BECKMAN PART NO. 580217

9/8/78

New Fuchsin pH=7
6 x 1 ml aliquots of Dye




DATE
ANALYST
SCAN SPEED
PERIOD
REF
SCALE
SAMPLE
UV
VIS
-.7
+.3

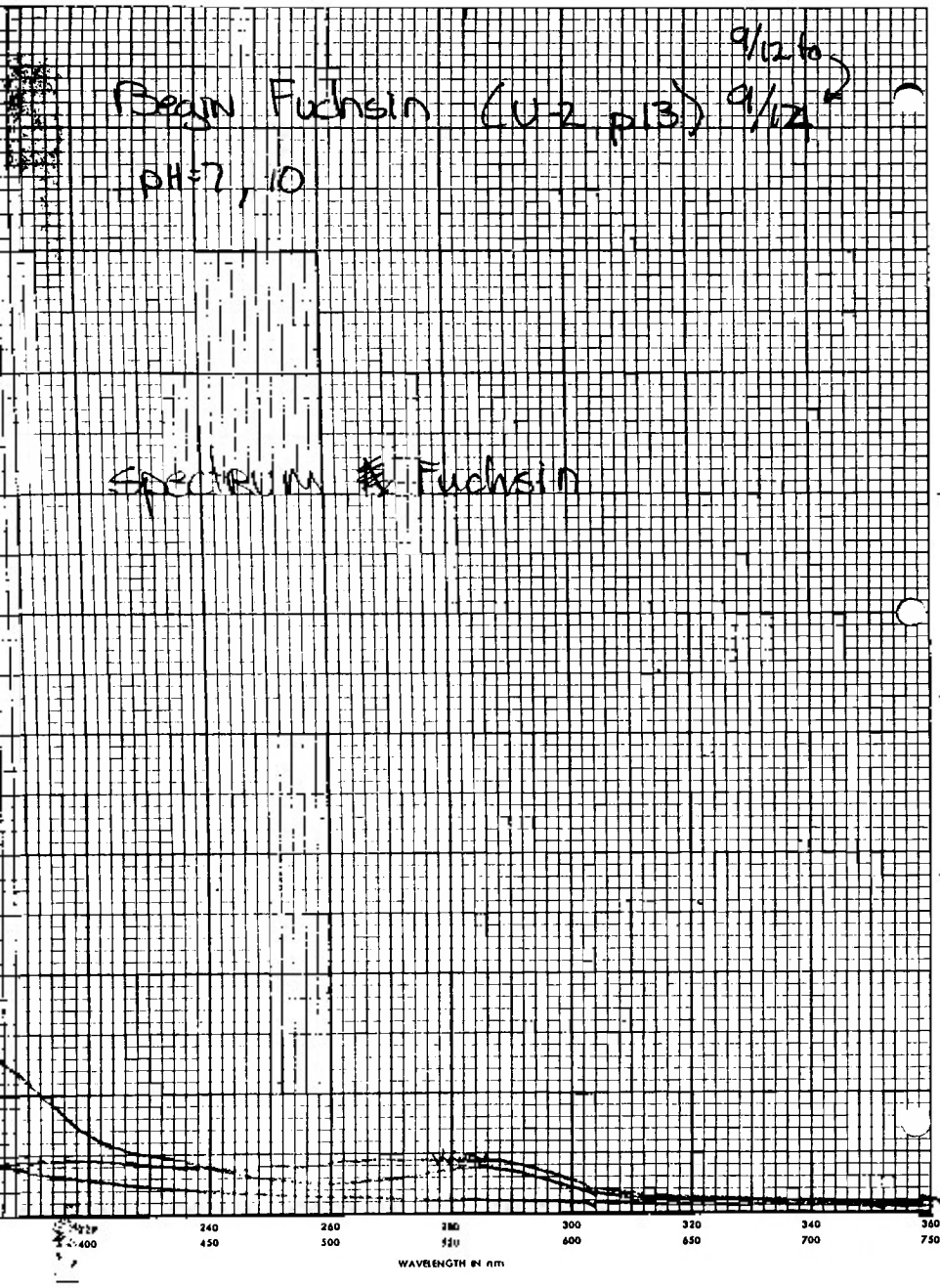
SPECTRUM OF FUCHSIN

BECKMAN INSTRUMENTS, INC. FULLERTON, CALIF. PRINTED IN U.S.A. REORDER BECKMAN PART NO. 580217

Begin Fuchsin (U-2 p13) 9/12 9/12b

pH=7, 10

Spectrum of Fuchsin

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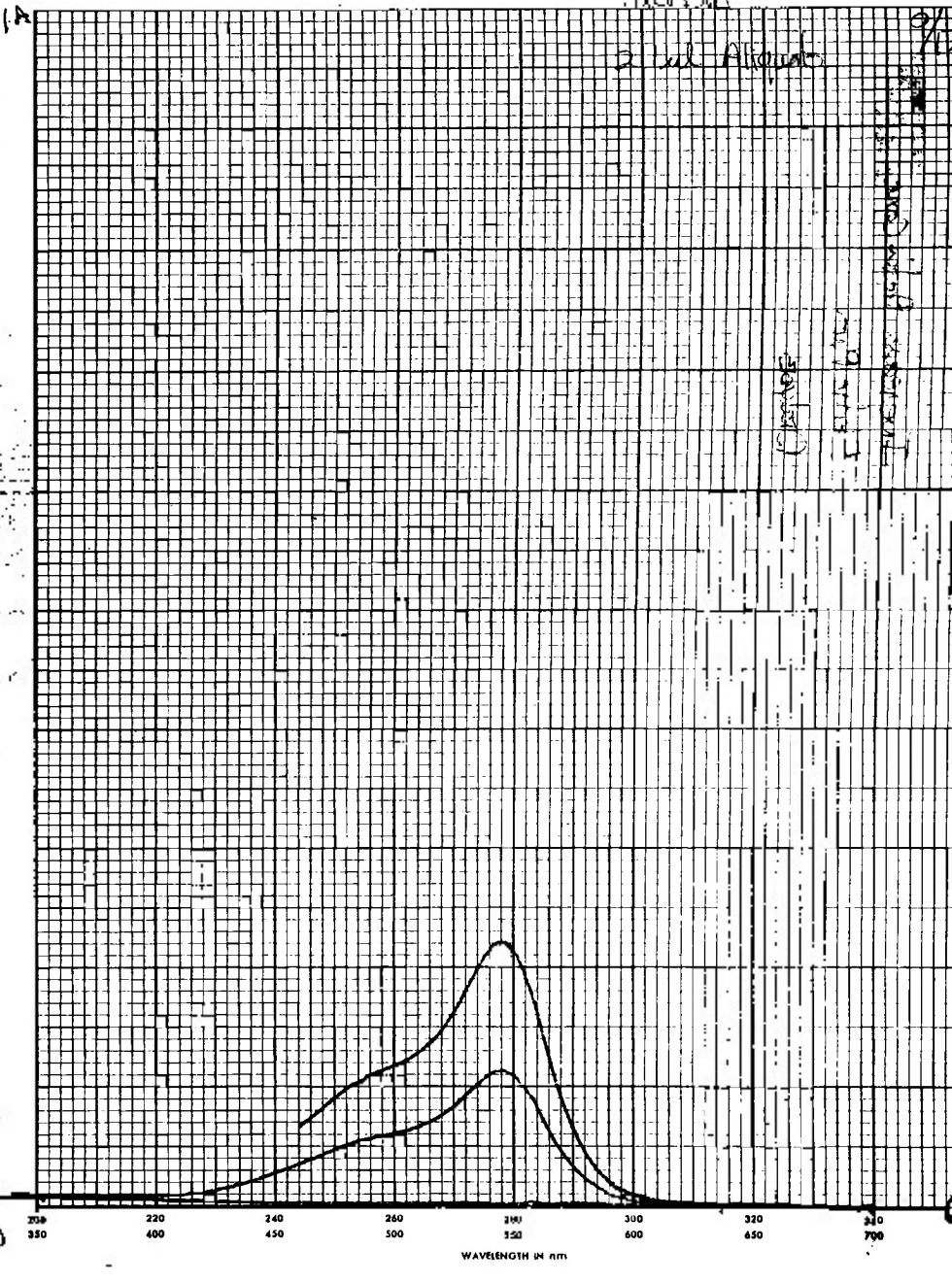
Fuchsin

2 ml Alcohol

9/



FUCHSIN - CRUDE PAPAIN DIFFERENCE SPECTRA

pH = 7

Fuchsine pH=7
Vis pH=7
Triple pH 1 & 13
WAVELENGTH IN nm
SAMPLE
REF
SCALE
SCAN SPEED
PERIOD
ANALYST
DATE
BECKMAN INSTRUMENTS, INC.
FULLERTON, CALIF.
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REORDER BECKMAN PART NO. 580217


UV pH=7 E
3,4
WAVELENGTH IN nm
BECKMAN INSTRUMENTS, INC.
FULLERTON, CALIF.
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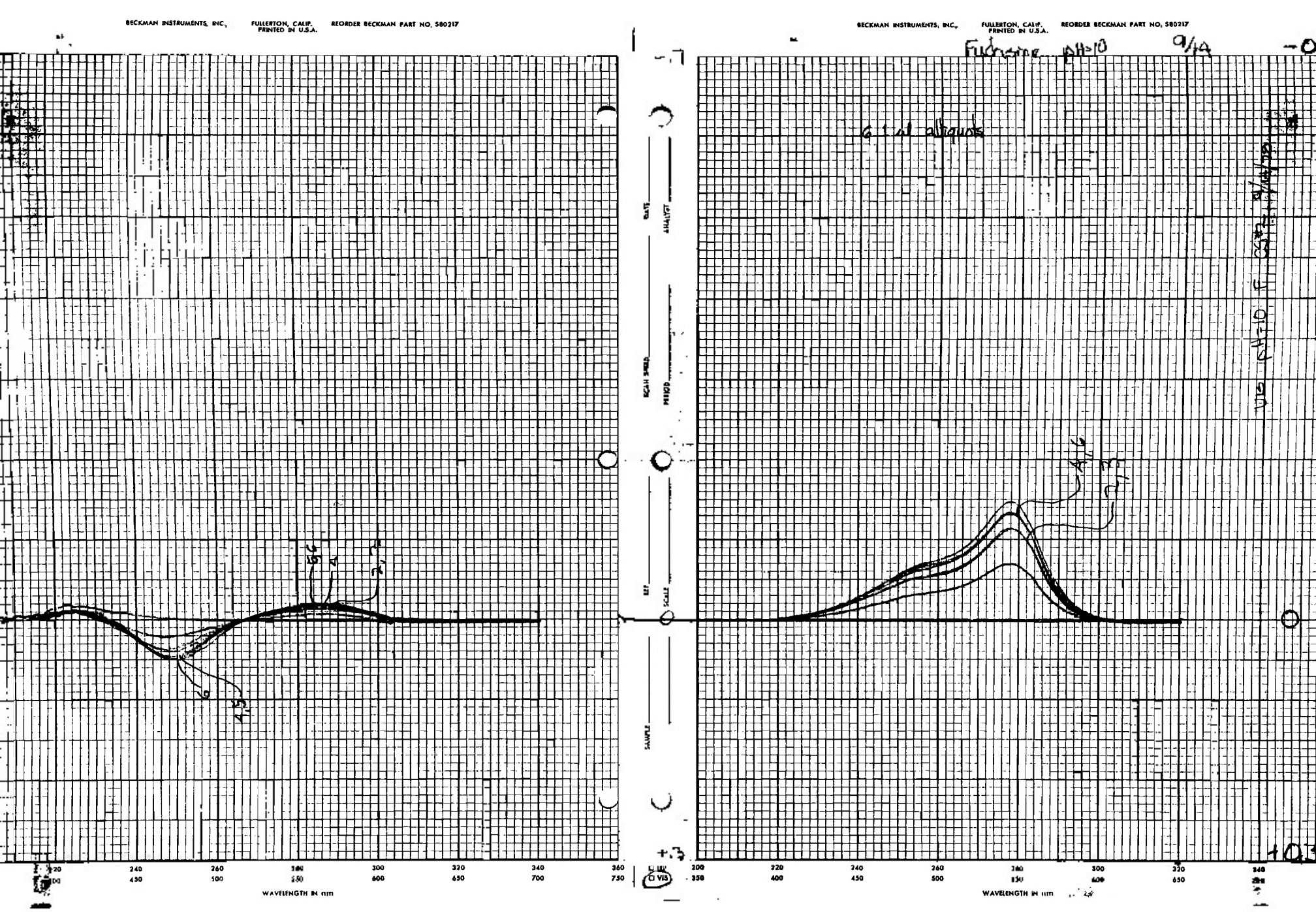
REORDER BECKMAN PART NO. 580217

FUCHSIN - CRUDE PAPAIN DIFFERENCE SPECTRA

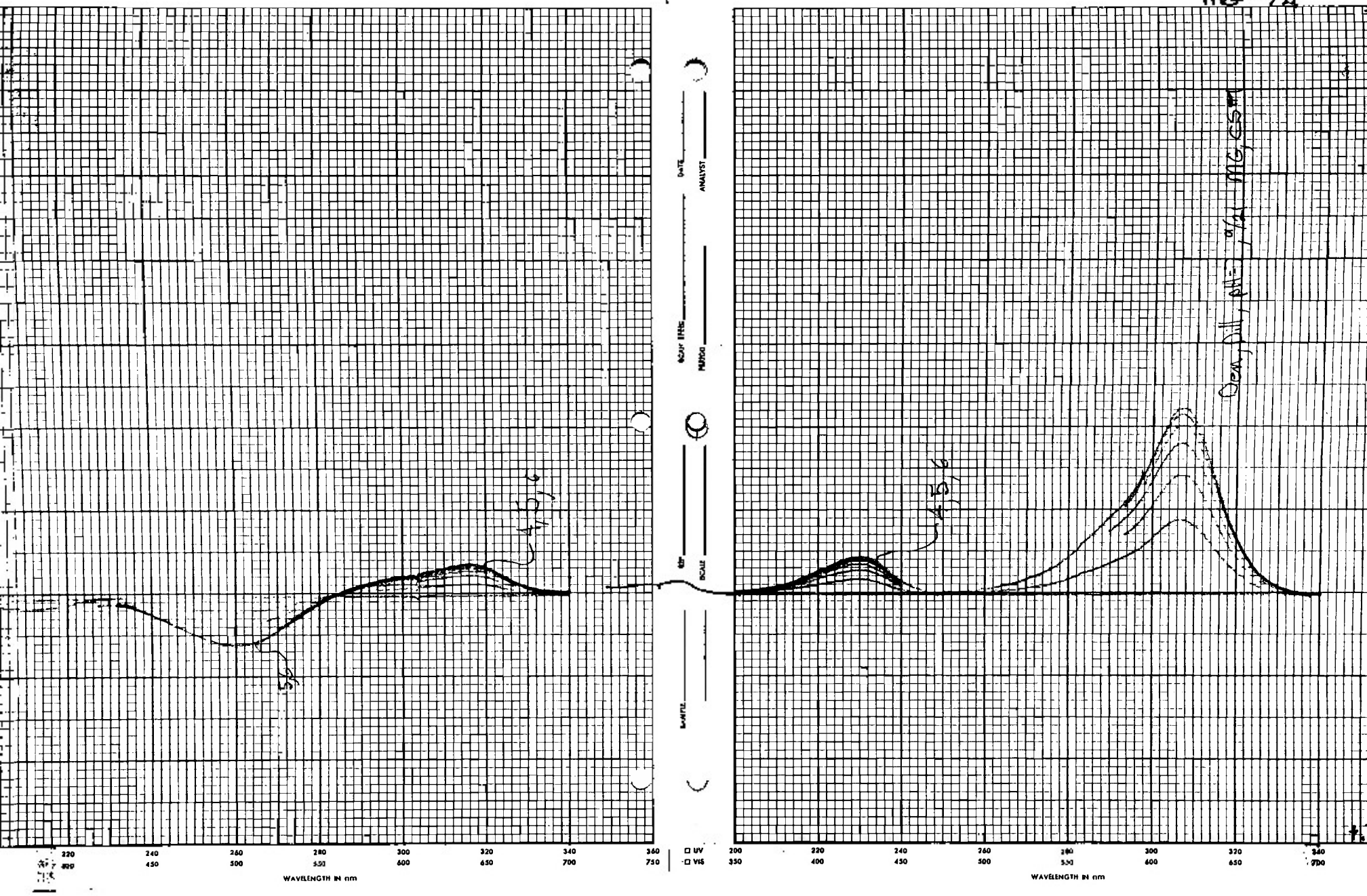
pH = 10


BECKMAN INSTRUMENTS, INC., FULLERTON, CALIF. REORDER BECKMAN PART NO. 580217
PRINTED IN U.S.A.
Fuchsine pH=10
9/4
6 1 ml aliquots
WAVELENGTH IN nm
WAVELENGTH IN nm
UV
VIS
RATE
ANALYST
SCAN SPEED
PERIOD
KEY
SCALE
SAMPLE

MALACHITE GREEN - DENATURED CRUDE PAPAIN DIFFERENCE SPECTRA

pH = 7

BECKMAN INSTRUMENTS, INC. FULLERTON, CALIF. PRINTED IN U.S.A. REORDER BECKMAN PART NO. 580217 86



MALACHITE GREEN - SIGMA PURIFIED PAPAIN DIFFERENCE SPECTRA

pH = 7

BECKMAN INSTRUMENTS, INC., FULLERTON, CALIF. PRINTED IN U.S.A. RECORDER BECKMAN PART NO. 580217 86

Cresol Red (100) 9/26 USP

pH 7

DATE ANALYST

SCAN SPEED PERIOD

REF SCALE

SAMPLE

□ uV □ VIS

WAVELENGTH IN nm

340 700 320 650 300 600 280 550 260 500 240 450 220 400 200 350

BECKMAN INSTRUMENTS, INC., FULLERTON, CALIF. PRINTED IN U.S.A. RECORDER BECKMAN PART NO. 580217 86

SPECTRA OF DARKEN (UV, pH 8) 9/29, 22

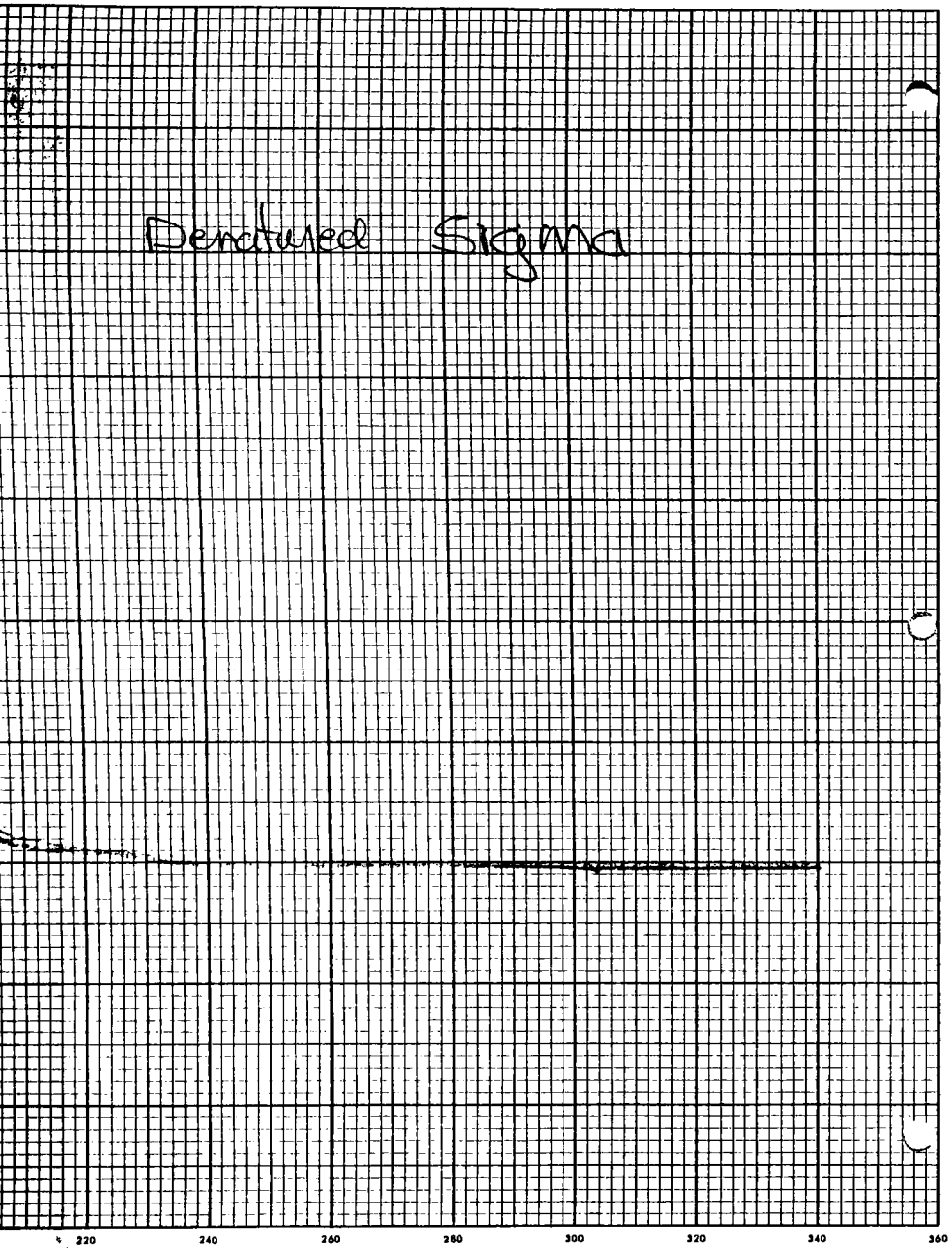
WAVELENGTH IN nm

340 700 320 650 300 600 280 550 260 500 240 450 220 400 200 350

MALACHITE GREEN - DENATURED SIGMA PURIFIED PAPAIN

DIFFERENCE SPECTRA

pH = 7



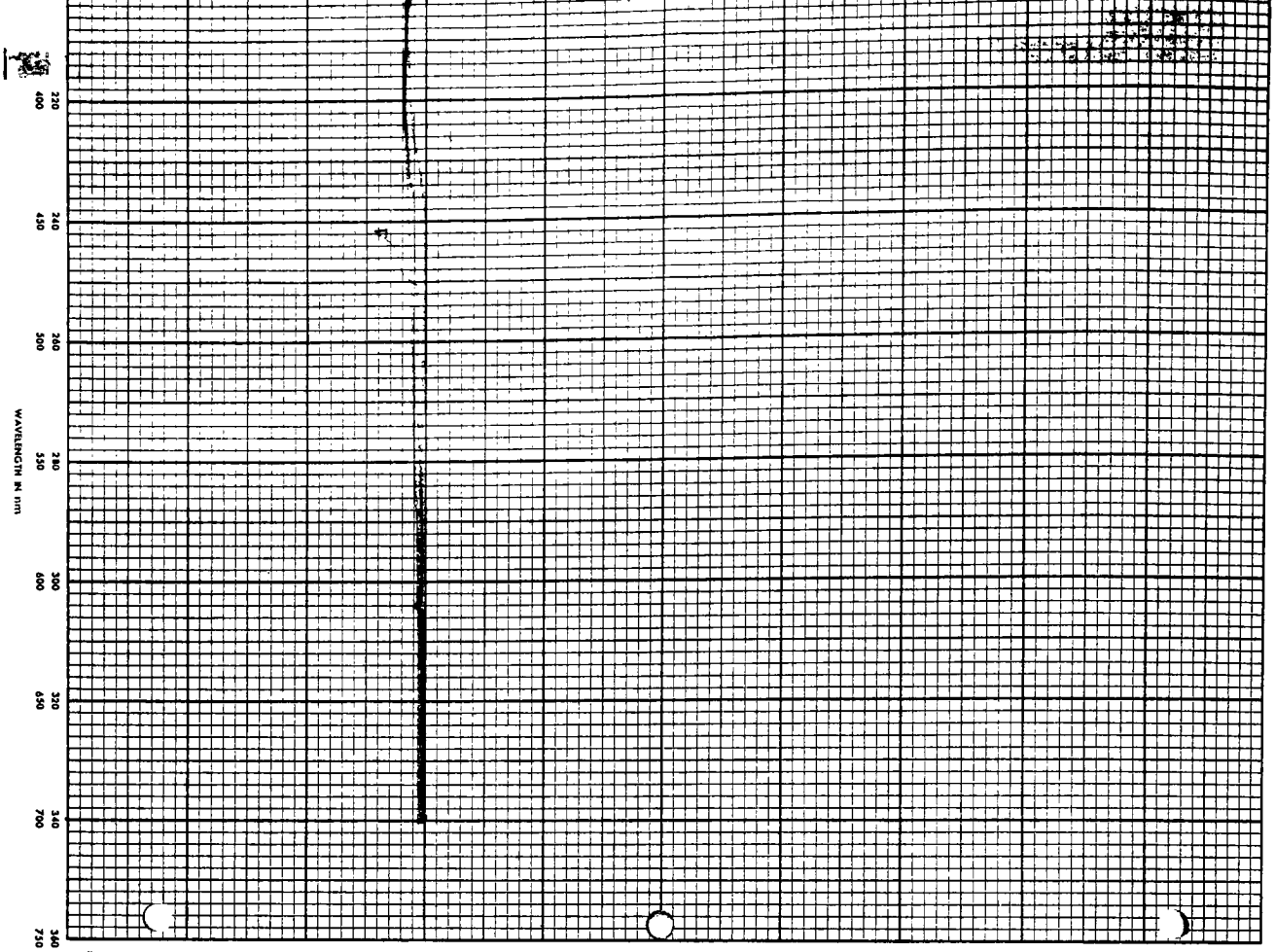

DATE ANALYST
SCAN SPEED PERIOD
REF SCALE
SAMPLE
□ UV
□ VIS

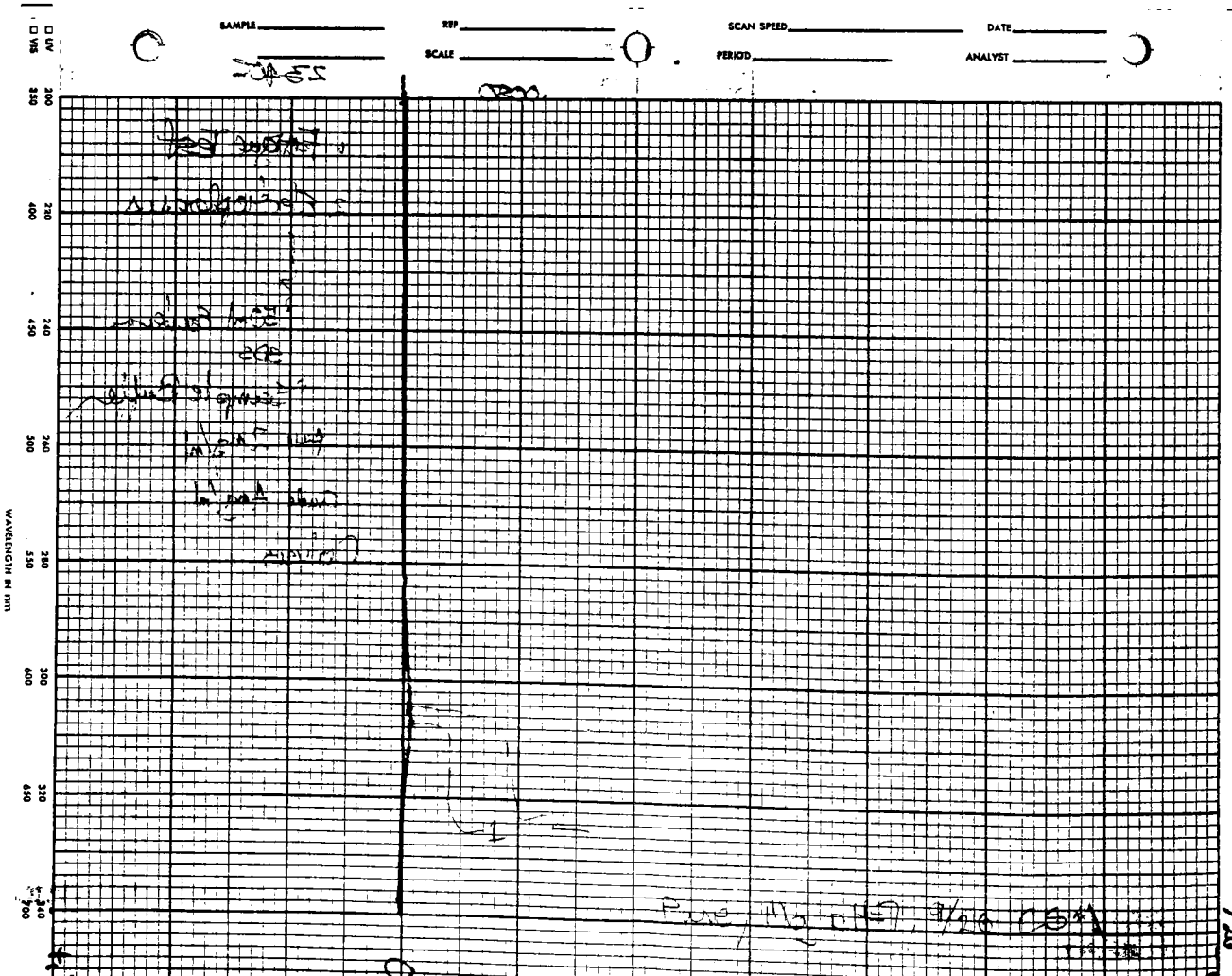
RECKMAN INSTRUMENTS, INC. FULLERTON, CALIF. PRINTED IN U.S.A. REORDER BECKMAN PART NO. 580217 86

MALACHITE GREEN - SIGMA PURIFIED PAPAIN DIFFERENCE SPECTRA

(Increased Concentration)

pH = 7


WAVELENGTH IN nm
220 240 260 280 300 320 340 360
400 450 500 550 600 650 700 750
BECKMAN INSTRUMENTS, INC.
FULLERTON, CALIF.
PRINTED IN U.S.A.
RECORDER BECKMAN PART NO. 580217
86


SAMPLE
REP
SCAN SPEED
DATE
SCALE
PERIOD
ANALYST
UV
VIS
WAVELENGTH IN nm
220 240 260 280 300 320 340 360
400 450 500 550 600 650 700 750
BECKMAN INSTRUMENTS, INC.
FULLERTON, CALIF.
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86

FUCHSIN - SIGMA PURIFIED PAPAIN DIFFERENCE SPECTRA

pH = 7

BECKMAN INSTRUMENTS, INC., FULLERTON, CALIF. PRINTED IN U.S.A. REORDER BECKMAN PART NO. 580217 86

ANALYST

DATE

SCAN SPEED

PERIOD

SCALE

REF

SAMPLE

UV

WAVELENGTH IN nm

ADE PEARLS FUCHSIN

BECKMAN INSTRUMENTS, INC., FULLERTON, CALIF. PRINTED IN U.S.A. REORDER BECKMAN PART NO. 580217 86

Basic Fuchsin

2.5 mg%/100ml

MALACHITE GREEN - WORTHINGTON PURIFIED PAPAIN

DIFFERENCE SPECTRA

pH = 7

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Malachite Green Worthington Papain
100 mg/100 ml

DATE
ANALYST
SCAN SPEED
PERIOD
REF
SCALE
SAMPLE

□ UV
□ VIS

200 220 240 260 280 300 320 340
350 400 450 500 550 600 650 700

WAVELENGTH IN nm

BECKMAN INSTRUMENTS, INC., FULLERTON, CALIF. PRINTED IN U.S.A. REORDER BECKMAN PART NO. 580217 86

MG 10/24

200 220 240 260 280 300 320 340
350 400 450 500 550 600 650 700

WAVELENGTH IN nm

NEW FUCHSIN - WORTHINGTON PURIFIED PAPAIN DIFFERENCE SPECTRA

pH = 7

NEW FUCHSIN - Whatman Paper
10/21 Whatman 1 Paper
0.1 M HCl
SAMPLE
REF
SCALE
SCAN SPEED
PERIOD
ANALYST
DATE
WAVELENGTH (nm)

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